Surgical Forum

Proceedings of The Forum Sessions
Forty-first Clinical Congress of the American College
of Surgeons, Chicago, Illinois
November, 1955

Surgical Forum

VOLUME VI

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AMERICAN COLLEGE OF SURGEONS
Chicago, Illinois, 1956

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Foreword

Beginning with this volume the American College of Surgeons has itself assumed responsibility for publication of the annual Surgical Forum. Minor alterations in format have been instituted. The same excellence of printing, binding and reproduction of illustrations that has characterized previous volumes has been maintained.

The Surgical Forum has come to be recognized as one of the most valuable, if not the most valuable, single surgical publication each year. In these volumes are recorded substantial portions of the current new surgical contributions of a fundamental nature. From year to year it is increasingly evident that these reports are not only expanding our basic knowledge but are also laying down the groundwork for forward strides in the field of clinical surgery. This volume should be available to all who are interested in surgical progress.

The grateful appreciation of the members of the Forum Committee is extended to Helene Coleman who has been responsible for editing the manuscripts for Volume VI of the Surgical Forum.

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Preoperative and Postoperative Care

INTRODUCTION

JAMES D. HARDY

The aim of supportive therapy is to improve the function of the various organs both individually and collectively. Accumulating evidence accentuates the awareness that the body organs function as a unit. The three chief coordinating systems which effect integration are the nervous system, the endocrine system, and the circulatory system. By the last is meant the general circulation of water, not merely that of blood; it is in water that nutrients are transported from the blood stream to the cells and that metabolites are transported from the cells to the body surfaces to be excreted.

Preoperative Preparation. It is an accepted fact that the depleted subject represents a less favorable operative risk than does the well nourished, well hydrated individual. Though many of the basic nutritional requirements fulfilled by previously empiric therapy have been identified, many remain obscure. Yet since it is desirable to bring the patient to operation in the best possible nutrition, it is important to know if and how he is depleted. McMurrey and his associates have worked out methods by which red cell volume, blood volume, total body water, extracellular water and total exchangeable chloride, sodium, and potassium may be measured simultaneously in the living subject. Such studies should provide information which will render preoperative replacement therapy more precise.

The intravenous administration of fat has continued to interest many workers. Unfortunately, most if not all of the preparations available commercially still give a 10 to 15 per cent reaction rate in human beings. The untoward effects consist chiefly of fever and, less often, chills. Many persons have wondered if the febrile response might not represent some innate effect of all fat when infused intravenously, but it appears that this may not be so. In a fine study, Payne and her co-workers have chemically separated beef fat into two fractions — Fraction A, which was white in color and highly lethal when infused in dogs, and Fraction B, a yellow oil which caused no reactions in dogs. The work suggests that the toxicity of fat may be dependent upon the length of the fatty acid chains and the degree of their saturation. Neutral fat of the body consists chiefly of long chains. If studies of this type can now make available a fat preparation that is safe for intravenous use, the long-sought abundant calorie source will be at hand. It has already been demonstrated that infused fat is metabolized by the body.

The Operation Itself. The patient is now ready for anesthesia — what agent shall be used? Apparently the metabolic response to different anesthetic agents varies markedly. It has been reported that ether causes a much

UROLOGY

EXCRETION OF ALDOSTERONE BY THE POSTOPERATIVE PATIENT*

BERNARD ZIMMERMANN, JAMES HUGH CASEY, HENRY S. BLOCH
EARL Y. BICKEL AND KALMAN COVRIK

That patients following major surgical procedures tend to exhibit strongly positive sodium balance and negative potassium balance has been well established. This phenomenon is of great importance in consideration of the management of electrolyte balance in postoperative patients and the hazards of administration of excess sodium chloride within the first few days after surgery is recognized by present-day surgeons. It is also generally agreed that this phenomenon involves participation of the endocrine system. That this and other aspects of the altered metabolism in the postoperative patient represents a physiologic form of hyperadrenalism has been logically suspected and confirmed by the finding of increased amounts of adrenal hormones in the blood and urine of postoperative patients. Although the hormones concerned with organic metabolism have been studied in some detail, little work has been directed toward elucidating the presence and mobilization of specific hormones concerned with electrolyte regulation beyond the suggestion that some mineralocorticoid resembling desoxycorticosterone might possibly be involved.[1]

In 1953 through the combined efforts of Simpson and Tait in England and Reichstein and his colleagues of Basel, a substance originally known as electrocortin, subsequently renamed aldosterone, was isolated from the so-called amorphous fraction of adrenal extracts. This material was found to have sodium retaining potency as high as 25 to 30 times that of desoxycorticosterone. It has also been found in urine and adrenal vein blood and is apparently mobilized under pathologic circumstances such as nephrosis, cirrhosis and congestive heart failure. In view of the high potency of this compound as a sodium retaining material, it appeared important to investigate its possible role in the postoperative surgical patient. In the present study, excretion of this material was measured in the urine of patients before and during the 24 hours after operation.

METHOD

Twenty-four hour urines were collected on ice without preservative and the conjugates hydrolyzed with beta glucuronidase. All values reported were done on enzymatically hydrolyzed urine although others have found higher values with acid hydrolysis.[3] Chloroform was removed and the residue redissolved in alcohol and subjected to partition between benzene and water. The aqueous phase was extracted again with chloroform and chromatograms were prepared in the propylene glycol-toluene system of Burton and Zaffaroni.[4]

Extracts from the same patient before and after operation were always run on the same chromatographic strip in order to eliminate possible variations which could be produced by day-to-day variations and physical

* From the Department of Surgery, University of Minnesota Medical School, Minneapolis, Minn. This work is supported in part by U. S. Public Health Service, Endocrinology Branch A-368 Endo (1), American Cancer Society Institutional Research Grant INSTR-49D and Minnesota Division, American Cancer Society.

greater stress response than does pentothal Nevertheless it does not necessarily follow that the latter is a safer anesthetic agent

The need for the restoration of an effective circulation following hemorrhage has continued to receive attention Serious question is being raised regarding the ultimate value of norepinephrine in the treatment of shock It is not enough to correct hypotension alone oligemic shock is more harmful than shock induced by hypotensive drugs or by high spinal anesthesia Blood loss by hemorrhage is to be treated with blood transfusion

The Postoperative Period Variations in plasma sodium concentrations and urine sodium excretion following major operations were described some time ago The plasma sodium level may fall even though less sodium is excreted in the urine Where then does the extracellular sodium go? Even though the volume of the extracellular space enlarges postoperatively the absolute sodium content does appear to diminish It had been thought that this sodium might temporarily have entered the bones but careful studies by Casey and Zimmerman appear to have ruled out this possibility

In a second but parallel study Zimmerman and Casey have shown that the postoperative decrease in sodium excretion is likely due to an increased secretion of aldosterone (electrocortin) by the adrenal cortex

Much other important work is being conducted and it is clear that the experimental method flourishes as an instrument for improving surgical care

to doses of desoxycorticosterone ranging from 0 to 50 μg. It was apparent from the results of these curves that aliquots of extracts should be used which would give values in the range corresponding to that of desoxycorticosterone in amounts less than 10 μg. In this range the relationship is nearly linear. Although the alcohol controls were carried out in connection with all the assays they are of questionable significance in experiments such as these where the urine of pre- and postoperative patients are measured simultaneously. Only 2 of 7 patients studied before operation had values of log K/Na which were greater than that of the alcohol controls. This indicates that, as has been previously demonstrated, many individuals do not excrete any aldosterone under basal circumstances. One individual (Fig. 2 number 1) actually exhibited material in the aldosterone fraction of the urine which caused slight sodium diuresis in the rat.

Figure 2 shows the pre- and postoperative values on 7 patients studied following major abdominal operations. It is apparent that in all instances values for the sodium retaining material in the urine were increased significantly after surgery with values of log K/Na reaching as high as 300 per cent of the preoperative amount. There appears to be no question that this sodium retaining factor is mobilized following surgery.

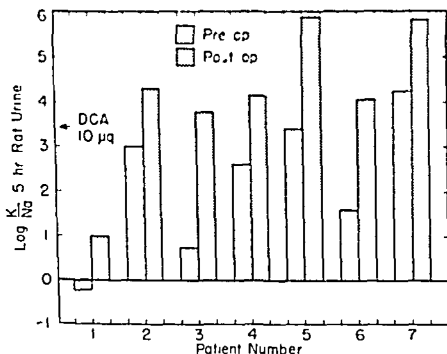


Fig. 2 Excretion of aldosterone before and after surgery in terms of log K/Na of the 5 hour rat urines

DISCUSSION

The finding of material with the chemical and pharmacologic characteristics of aldosterone in the urine of postoperative patients would appear to be a significant step in the search for an endocrine basis for the sodium retention which occurs after surgery. A theoretical problem of considerable interest however arises in explaining the mobilization of this material. Luetscher and others have shown that ACTH does not stimulate the pro-

factors which alter the development of chromatograms Portions of the chromatographic strip were treated with antimony trichloride to produce color reactions and using the bands thus produced as a guide along with the position of pure compounds E and F the area of the paper representing aldosterone which is slightly more polar than compound E was eluted with alcohol The alcohol eluates were then assayed in adrenalectomized rats The rats were used 4 days following adrenalectomy and were maintained on saline drinking water until 12 hours before the runs Each rat was given an aliquot of extract corresponding to the urine excreted by the patient in 20 minutes Control rats were given pure alcohol in the same amount as that used as a solvent for the extract (1 cc) Desoxycorticosterone standards were given in the same amount of alcohol Ten adrenalectomized rats were included in each group including pre and postoperative extracts desoxycorticosterone standards and alcohol controls so that a total of 40 rats were required for each experiment The urine excreted by the rats for a 5 hour period after the injection was collected in beakers The rats were kept on a metal screen in the top of the beaker during the assay period

In preliminary experiments values of sodium and potassium and sodium to potassium ratio in 5 hour rat urines were related to varying dosages of desoxycorticosterone Trial of various methods of calculation revealed that the log K/Na was the most critical quantity and related in the most nearly linear fashion to standardization doses of desoxycorticosterone This method of assay and calculation is similar to that which has been described by Johnson[5] Figure 1 illustrates a standardization curve relating log K/Na

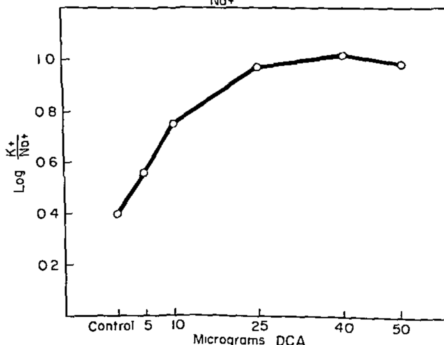


Fig 1 Electrolyte excretion of adrenalectomized rats in response to various dosages of desoxycorticosterone acetate expressed as logarithm K/Na

to doses of desoxycorticosterone ranging from 0 to 50 μg. It was apparent from the results of these curves that aliquots of extracts should be used which would give values in the range corresponding to that of desoxycorticosterone in amounts less than 15 μg. In this range the relationship is nearly linear. Although the alcohol controls were carried out in connection with all the assays they are of questionable significance in experiments such as these where the urine of pre- and postoperative patients are measured simultaneously. Only 2 of 7 patients studied before operation had values of log K/Na which were greater than that of the alcohol controls. This indicates that, as has been previously demonstrated, many individuals do not excrete any aldosterone under basal circumstances. One individual (Fig. 2, number 1) actually exhibited material in the aldosterone fraction of the urine which caused slight sodium diuresis in the rat.

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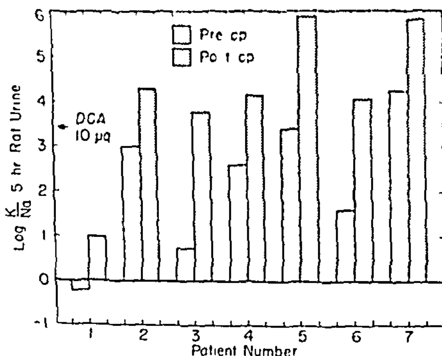


Fig. 2. Excretion of aldosterone before and after surgery in terms of log K/Na of the 5 hour rat urines.

DISCUSSION

The finding of material with the chemical and pharmacologic characteristics of aldosterone in the urine of postoperative patients would appear to be a significant step in the search for an endocrine basis for the sodium retention which occurs after surgery. A theoretical problem of considerable interest, however, arises in explaining the mobilization of this material. Luetscher and others have shown that ACTH does not stimulate the pro-

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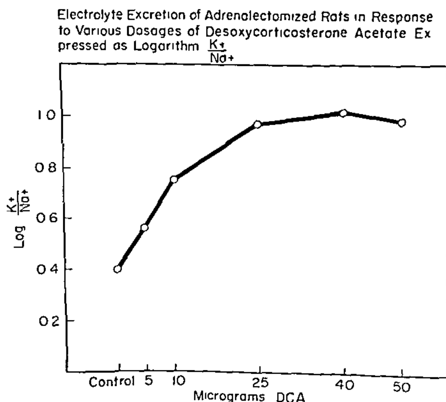


Fig 1 Electrolyte excretion of adrenalectomized rats in response to various dosages of desoxycorticosterone acetate expressed as logarithm K/Na

THE ROLE OF BONE SODIUM IN SURGERY*

JAMES HUGH CASEY AND BERNARD ZIMMERMANN

Certain aspects of sodium metabolism cannot be described by overall balance studies. Unlike potassium in which the daily output ordinarily equals input in the normal state, sodium balance may be either positive or negative over short term periods and zero balances are obtained only when longer time intervals are considered. In addition certain discrepancies occur under pathologic circumstances when balance data are compared with changes in total extracellular sodium. One such discrepancy occurs in the early postoperative period during which serum sodium levels often paradoxically fall in the face of positive sodium balance as measured by records of intake and output[1]

Harrison, Darrow, and Yannet[2] discovered that over one half of the large quantity of skeletal sodium is in excess of that in solution in the extracellular fluid of bone. This excess bone sodium is osmotically inactive, insoluble in water and strong alkalies but soluble in acid and is probably part of an apatite complex similar to the sodium found in naturally occurring fluorapatites. It has been suggested that this pool of sodium within the body is an active reservoir to be called upon in deficiency states and that at least a portion of the excess bone sodium of the laboratory animal is available to the extracellular fluid under such circumstances[3] That fluctuations in this reservoir may be of importance in some of the above mentioned discrepancies which are seen clinically in patients has been proposed by Moore[4]

METHOD

In order to investigate more fully the role of bone sodium in the surgical patient, electrolyte balance studies were performed in 17 patients undergoing pulmonary resection. The electrolyte composition of the resected ribs was determined and compared to that of ribs biopsied on the second postoperative day. The cortex of the rib sections was mechanically separated from the marrow and dried for 18 hours at 105 C. Weighed aliquots of the dried cortical bone were dissolved in nitric acid and the calcium was removed by three successive precipitations with oxalic acid in alkaline solution after the method of Bergstrom and Wallace[5] With this method co-precipitation of sodium has been no problem. Chloride concentration was determined by amperometric titration with silver nitrate, a method similar to that described by Kolthoff[5] When this method of rib analysis was standardized on known mixtures of sodium, potassium and calcium salts in the approximate proportion found in bone, accuracy in these determinations was found to be within 1.5 per cent. The composition of different ribs removed from any one individual were found to be identical within the same range of error when thoracoplasty or autopsy material was studied.

The extra bone sodium (the difference between the total bone sodium

* From the Department of Surgery, University of Minnesota and Thoracic Service, Anoka State Hospital. Supported in part by the Minnesota Division, American Cancer Society and U. S. Public Health Service, Endocrinology Branch, A 368 Endo (1).

duction of aldosterone from the adrenal. A low sodium diet, on the other hand, does cause aldosterone output in human subjects[6]. It appears therefore that the production of aldosterone by the adrenal is attuned to the necessity for sodium retention but is independent of pituitary corticotrophic activity. Why then should this steroid be mobilized after surgery? A possible explanation is that serum sodium following surgery tends to be low and this itself may be the critical factor for aldosterone production. Experiments to evaluate this possibility are in progress. The experiments reported here refer only to the first 24 hours after surgery. Studies currently in progress, however, are designed to follow aldosterone excretion throughout the postoperative course and relate it to the balance of sodium and potassium, to the extracellular levels of these ions, and to the excretion of 17-hydroxycorticoids in order to ascertain whether this substance is more closely related to the actual duration of positive sodium balance than other endocrine materials which have already been measured. In this connection it is of interest that Venning[7] who studied patients at a somewhat later period after trauma found no increase in aldosterone. Llaurado, on the other hand, has recently reported findings very similar to ours[8].

SUMMARY

In 7 patients following major surgical operations the excretion of steroid material with chemical, chromatographic, and physiologic properties of aldosterone has been found to be greatly increased over preoperative values. The significance of this fact and its relationship to the degree of positive sodium balance which normally follows operations is discussed.

REFERENCES

1. Johnson, H. T., Conn, J. W., Iob, V., and Coller, F. A.: Postoperative salt retention and its relation to increased adrenal cortical function. Ann. Surg. *132*: 374, 1950.
2. Simpson, S. A., Tait, J. F., Wettstein, A., Neher, R., v. Euw, J., and Reichstein, T.: Isolierung eines neuen kristallisierten Hormons aus Nebennieren mit besonders hoher Wirksamkeit auf den Mineralstoffwechsel. Experientia, Basel *10*: 132, 1954.
3. Luetscher, J. A., and Johnson, B. B.: Chromatographic separation of the sodium retaining corticoid from the urine of children with nephrosis compared with observations on normal children. J. Clin. Invest. *33*: 276, 1954.
4. Burton, R. B., Zaffaroni, A., and Keutman, E. H.: Paper chromatography of steroids. II. Corticosteroids and related compounds. J. Biol. Chem. *188*: 763, 1951.
5. Johnson, B. B.: Bioassays of adrenal cortical steroids on the basis of electrolyte excretion by rats. Effects of 11 desoxy and 11 oxy steroids. Endocrinology *54*: 196, 1954.
6. Luetscher, J. A., Jr., and Axelrad, B. J.: Increased aldosterone output during sodium deprivation in normal men. Proc. Soc. Exp. Biol., N. Y. *87*: 650, 1954.
7. Venning, E. H., Beck, J. C., Dyrenfurth, I., and Giroud, C. J. P.: Studies on the excretion of the sodium retaining corticoid. J. Clin. Endocr. Metab. *15*: 855, 1955.
8. Llaurado, J. G.: Increased excretion of aldosterone immediately after operation. Lancet, Lond. *269*: 1295, 1955.

immediately following surgery presented positive sodium balance in some cases.

It has been estimated that bone makes up 16 per cent of body weight. However, water and marrow make up a great deal of this bone mass. Ribs lose approximately 4[illegible] per cent of their weight when water and marrow are excluded. Consequently, for purposes of analyzing bone sodium changes a figure of 9 per cent is more realistic for the percentage of body weight constituted by the skeleton. Changes in total bone sodium as projected from changes in rib sodium were calculated. While the electrolyte content of different bones in the body varies somewhat, the rib is intermediate in composition and such projection appears reasonable. In all instances bone sodium variation between the time of thoracotomy and the time of biopsy 2 days later were slight and could not account for the discrepancies in the balance studies (Fig. [illegible])

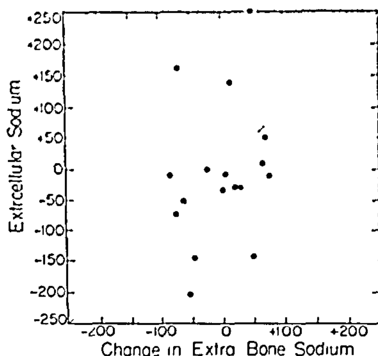


Fig. 2. Changes in extra bone sodium as related to comparison of balance data with total amount of sodium in the extracellular fluid.

Ordinate shows actual state of sodium balance in milliequivalents on the second postoperative day greater or smaller than expected from analysis of the change in serum sodium levels alone.

Abscissa shows changes in total body extra bone sodium in milliequivalents. Dotted line shows theoretical regression line if bone sodium actually acted as an active sodium reservoir. Changes in bone sodium would then compensate for the observed discrepancies.

To investigate further the role of bone in sodium metabolism, the ribs of 10 patients undergoing mitral commissurotomy were analyzed. Patients with compensated rheumatic heart disease are known to have increased exchangeable sodium values[8] even in the presence of normal or slightly depressed serum sodium levels. This has suggested a hidden sodium storehouse. However, rib electrolyte values were in the normal range relative

per kilogram dry cortical bone and the sodium present in solution in the extracellular fluid of bone) is calculated from the following formula

$$\frac{\text{Chloride/kg bone}}{(Cl^-)_E} \times (Na^+)_F = \text{extracellular sodium/kg of bone}$$

Total sodium content of 1 kg bone minus extracellular sodium in 1 kg of bone equals extra bone sodium in mEq /kg

Calculation of the extracellular fluid of bone depends on the assumption of the exclusively extracellular position of chloride Values for ribs in balance study are shown in Figure 1

Case number	Day of surgery		2nd post-op day		% change in total bone sodium	% change in extra bone sodium	Change in extra bone sodium mEq/kg
	Total rib sodium meq/kg	Extra bone sodium meq/kg	Total rib sodium meq/kg	Extra bone sodium meq/kg			
1	300	271	286	260	470%	40%	-11
2	304	271	308	273	[illegible]	+74	+2
3	304	263	312	276	26%	49%	+13
4	313	280	298	267	48%	46%	-13
5	302	271	292	260	33	41%	-11
6	308	277	312	285	13%	+29%	+12
7	296	262	297	266	34%	+15%	+4
8	308	278	291	263	55%	-54%	15
9	302	264	293	259	3%	-2%	5
10	297	263	305	272	+27%	+34%	+9
11	301	271	292	260	3%	41%	-11
12	287	252	289	257	7%	+2%	+5
13	291	254	295	262	137%	+32%	+8
14	303	268	286	253	-56%	-56%	15
15	301	266	300	267	33%	38%	+1
16	310	270	310	270	0%	0%	0
17	301	270	292	260	3%	37%	10

Fig 1 Individual rib sodium values in 17 thoracic surgical patients

As is well known the sodium content of bone increases with age both absolutely and proportionately as a result of decreased water content In the adult human ribs analyzed by the above method had an excess sodium content varying from 250 to 285 mEq per kg Chloride varied from 20 to 30 mEq and potassium from 9 to 15 mEq

In the 17 patients studied extracellular sodium was calculated from serum sodium and the extracellular volume with appropriate corrections for the Gibbs Donnan effect For the extracellular volume the value of 20 per cent of body weight was used since previous work from this laboratory had indicated that in the first 2 days after similar operations major changes in the thiocyanate and thiosulfate spaces did not occur[6] By comparing total extracellular sodium with the sodium intake and output records the extent of agreement between serum sodium and external losses could be ascertained By such consideration 10 patients exhibited serum sodium levels on the second postoperative day which were compatible with the balance data but 3 patients apparently lost more sodium as determined by serum levels than could be accounted for by output and in 4 patients serum levels dropped less than expected in view of losses

Since balance studies were carried on over only the first 2 postoperative days the frequently high urine sodium values during the 12 to 24 hours

immediately following surgery presented positive sodium balance in some cases.

It has been estimated that bone makes up 16 per cent of body weight. However, water and marrow make up a great deal of this bone mass. Ribs lose approximately 45 per cent of their weight when water and marrow are excluded. Consequently, for purposes of analyzing bone sodium changes a figure of 9 per cent is more realistic for the percentage of body weight constituted by the skeleton. Changes in total bone sodium as projected from changes in rib sodium were calculated. While the electrolyte content of different bones in the body varies somewhat, the rib is intermediate in composition and such projection appears reasonable. In all instances bone sodium variation between the time of thoracotomy and the time of biopsy 2 days later were slight and could not account for the discrepancies in the balance studies (Fig. 2).

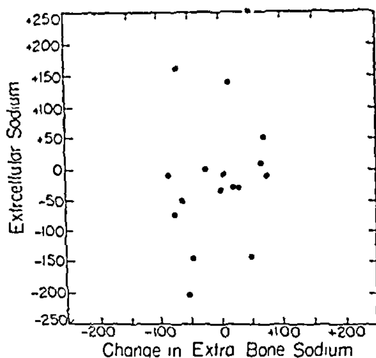


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to age in all 10 patients Strict observance of a low salt diet for some months preoperatively also had no effect on the bone sodium values

Whether sudden loads of sodium may be stored temporarily in bone and subsequently released to the body as needed is an interesting question To test this premise 3 patients were given large infusions of saline for 12 hours immediately prior to thoracotomy One patient received 18 gm of sodium chloride and 2 patients received 27 gm of sodium chloride Urine sodium during the same period was measured and subtracted from the amount infused to calculate the actual amount of sodium retained in the body One hundred twenty five, 201 and 178 mEq respectively were retained However the sodium content of the ribs subsequently removed was not increased over the normal range for the age of the patient by such sodium loads

These experiments suggest that the sodium in the crystal lattice apatite structure of bone is relatively stabile and not subject to ready motility in response to short term requirements of the body However over longer periods of time certain electrolyte deficiencies due to chronic illness may be in part compensated for by bone sodium Four out of 5 patients with chronic illness and low serum sodium values had rib sodium values which were less than normal at postmortem examination The mechanism of bone sodium turnover in such cases is probably related to the normal process of bone resorption and repair

Potassium is present in very small amounts in marrow free bone Even during long term potassium deficiency states bone cannot be relied upon to supplement body potassium stores

Emphasis is placed on the exclusive use of marrow free ribs for all experiments If the large mass of marrow in ribs was not removed prior to analysis any change in the electrolyte composition of marrow cells and water would be erroneously ascribed to bone

CONCLUSIONS

1 Discrepancies arising from the comparison of sodium intake and output with serum sodium levels in the early *postoperative patient cannot* be explained by changes in bone sodium

2 Patients with compensated rheumatic heart disease have normal bone sodium values

3 Massive infusions of saline did not increase the extra bone sodium values above the normal ranges

4 Over longer periods of time bone sodium may partially alleviate sodium deficient states

5 The exclusive use of marrow free rib for analysis is emphasized

REFERENCES

1 Moore F D and Ball M R The metabolic response to surgery Springfield Ill Charles C Thomas 1952

2 Harrison H E Darrow D C and Yannet H Total electrolyte content of animals and its probable relation to the distribution of body water J Biol Chem *113* 515 1936

3 Bergstrom W H and Wallace W M Bone as a sodium and potassium reservoir J Clin Endocr *33* 867 1954

4 Moore F D Bone sodium Ann Surg *139* 253 1954

5 Kolthoff I M and Kuroda P K Argentometric amperometric titration of traces of chloride Analytical Chem *23* 1306 1951

6. Delaney, H., Mowlem, A., and Zimmermann, B. The effect of surgery on the extracellular water and electrolytes. In Surgical Forum IV. Philadelphia: W. B. Saunders Co., p. 40, 1953.
7. Bischoff, E. Einige Gewichts und Trocken Bestimmungen der Organe des menschlichen Koerpers. Ztschr. f. rat. med. 20: [illegible], 1863.
8. Moore, F. D., Edelman, I. S., Olney, J. M., James, A. H., Brooks, L., and Wilson, G. M. Body sodium and potassium. III. Interrelated trends in alimentary, renal and cardiovascular disease; Lack of correlation between body stores and plasma concentration. Metabolism 3: 334, 1954.

FURTHER STUDIES IN BODY FLUID PHYSIOLOGY I. THE EFFECT OF 3% NaCl, M/6 Na LACTATE AND M/6 NH_4Cl ON PLASMA CARBON DIOXIDE COMBINING POWER, pCO_2 AND BLOOD pH*

EDDIE EUGENE BRAMLITT AND JAMES D. HARDY

That pulmonary function in buffering infused acid or alkali by the excretion or retention of carbonic acid is significant has recently been questioned. The purpose of this study was to re-examine the effect of intravenous ammonium chloride, sodium lactate, and hypertonic saline solutions upon the acid-base equilibrium of the blood. The changes in carbon dioxide combining power (CO_2) which follow the infusion of alkalinizing or acidifying solutions are well known, but for critical appraisal of acid-base equilibrium it is desirable to measure changes in blood pH and pCO_2. These values, not commonly determined, reflect the effectiveness of rapid changes in carbonic acid content of the blood in preventing shifts in blood pH before the more leisurely renal excretion of fixed acid or base, as necessary, has had time to occur.

METHODS AND PROCEDURE

Fifteen patients convalescing from relatively minor illnesses were chosen at random from the wards of the John Gaston Hospital. For the most part these patients were in normal fluid, electrolyte and acid-base balance. They were divided into 3 groups of 5, and each group was used to evaluate 1 of the 3 solutions.

One liter of the appropriate solution was administered intravenously to each patient over a 90 minute period. Measurements of the venous pH, pCO_2 (representing carbonic acid), and carbon dioxide combining power (CO_2) were performed 1 hour before the infusion was started, again when it was half completed, and at the end of the infusion. Arterial samples were not drawn.

Measurements of pH values were made on whole blood in a Beckman potentiometer (Model G) using a glass electrode. With our instrument the normal values varied from 7.49 to 7.56. While the latter figure is somewhat

*From the Department of Surgery and Surgical Laboratories, Medical College of the University of Tennessee, and the John Gaston Hospital, Memphis, Tennessee. This work done under Army Contract No. DA 49 007 MD 296.

to age in all 10 patients Strict observance of a low salt diet for some months preoperatively also had no effect on the bone sodium values

Whether sudden loads of sodium may be stored temporarily in bone and subsequently released to the body as needed is an interesting question To test this premise 3 patients were given large infusions of saline for 12 hours immediately prior to thoracotomy One patient received 18 gm of sodium chloride and 2 patients received 27 gm of sodium chloride Urine sodium during the same period was measured and subtracted from the amount infused to calculate the actual amount of sodium retained in the body One hundred twenty five 201 and 178 mEq respectively were retained However the sodium content of the ribs subsequently removed was not increased over the normal range for the age of the patient by such sodium loads

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RESULTS AND DISCUSSION

The data are tabulated in Tables 1 to 3

Response of pH, pCO, and CO_2 to 1/6 M Sodium Lactate Intravenously The average rise in blood pH was from 7 51 to 7 60 It was noted that this change had occurred within the first 15 minutes of the infusion and the pH did not alter appreciably after that time In 2 patients (C J and T H) the pH was actually higher after 15 minutes than after completion of the infusion possibly due to an inconstant rate of infusion The average change in pCO was from 46 5 to 52 5 mm of Hg pressure During the first 15 minute period however little change was noted Most of the increase occurred during the last half of the infusion and this too might have had an important influence upon the failure of the pH to rise further after 15 minutes The CO_2 increased steadily throughout the infusion beginning at an average of 29 6 and ending at an average of 31 8 mEq /liter

In analyzing these changes it would appear that the buffer systems responded sluggishly at the beginning of the infusion permitting the blood pH to rise The decreased hydrogen ion concentration then stimulated the chemoreceptors and carbonic acid was retained by virtue of diminished pulmonary ventilation-compensation by means of the respiratory bicarbonate buffer system During the last half of the infusion a sudden increase in the pCO_2 value (representing a respiratory retention of carbon dioxide) enabled the $BHCO_3/H_2CO_3$ ratio (Henderson Hasselbalch equation) to return toward normal with a resulting leveling off of the pH Of course the kidneys must finally excrete the infused sodium excess

Response of pH, pCO and CO to 1/6 M Ammonium Chloride The average change in blood pH following administration of this solution was from 7 56 to 7 50 almost the opposite of the response to 1/6 M sodium lactate The pCO_2 decreased from 42 5 to 35 2 mm Hg and the CO correspondingly declined from 29 8 to 21 6 mEq /liter

Although average values were almost reciprocal the response to ammonium chloride was not as uniform as to sodium lactate This may well have been due to the gastro intestinal disturbances which accompanied the administration of intravenous ammonium chloride Four of the 5 patients who received NH_4Cl became acutely ill with pronounced nausea and copious vomiting The fifth became only mildly nauseated This complication was not anticipated since the infusion of ammonium chloride in alkalotic individuals rarely causes such symptoms

Response of pH pCO, and CO to Hypertonic Saline 3 Per Cent Intravenously There was little change following administration of this solution The pH changed from 7 51 to 7 52 the pCO from 17 5 to 17 6 mm Hg and the CO from 31 to 29 1 mEq /liter All of these variations were within the limits of error This lack of change was somewhat surprising for it is well established that prolonged infusion of NaCl will result in a degree of acidosis

SUMMARY AND CONCLUSIONS

Blood pH pCO_2 and CO values have been used to illustrate the effectiveness of the blood buffer systems in preventing a serious change of pH following the infusion of acid or alkaline solutions in relatively large amounts over a comparatively short period of time The pCO_2 increased

higher than that usually reported the results should be subject to valid comparison of data obtained from the same individual The Roughton Scholander microsyringe method for the determination of blood gases was used for measuring the pCO_2 the normal value for venous blood is a pressure of approximately 46 mm Hg The CO_2 values were determined by the routine Van Slyke volumetric method with a normal of approximately 27 mEq/liter In order to minimize variations due to technique all determinations were performed by the same technician

Table 1 1 Liter Sodium Lactate

			pH			pCO_2 in mm Hg			CO_2 in mEq			
NAME	SEX	AGE	B	D	A	B	D	A	B	D	A	ILLNESS
C J	M	41	7.58	7.63	7.60	46.86	47.68	51.25	28.0	31.0	35.0	Stabbed & multiple cuts
T H	M	73	7.52	7.63	7.58	46.86	49.33	48.50	29.0	31.0	32.0	Rt indirect ing hernia
R B	M	23	7.49	7.57	7.58	41.87	41.10	51.75	33.0	30.0	37.0	Felon
P S	F	22	7.53	7.62	7.65	41.93	41.93	51.26	29.0	35.0	35.0	Appendectomy
S T	M	27	7.49	7.54	7.58	55.08	46.04	50.15	29.0	33.0	35.0	Acute appendicitis
AVERAGES			7.54	7.60	7.60	46.52	45.21	52.58	29.6	32.6	34.8	

B—Before D—During A—After

Table 2 1 Liter Ammonium Chloride

			pH			pCO_2 in mm Hg			CO_2 in mEq			
NAME	SEX	AGE	B	D	A	B	D	A	B	D	A	ILLNESS
G J	M	25	7.59	7.63	7.51	50.64	36.17	31.24	32.0	29.0	26.0	Stabbed (Nausea & Vomiting)
F H	M	35	7.53	7.54	7.47	44.06	40.99	46.04	33.0	31.0	28.0	Perirectal Abscess (Slightly sick)
G B	M	75	7.55	7.49	7.44	39.46	37.19	32.06	29.0	25.0	20.0	Ing Hernia (Nausea & Vomiting)
C B	M	48	7.56	7.46	7.49	39.46	34.58	29.60	27.0	25.0	23.0	Hernia (Nausea & Vomiting)
T W	M	47	7.56	7.52	7.57	38.64	34.53	37.01	28.0	27.0	26.0	Ulcer (Nausea & Vomiting)
AVERAGES			7.56	7.54	7.50	42.45	36.69	35.19	29.8	27.4	24.6	

B—Before D—During A—After

Table 3 1 Liter 3% Saline

			pH			pCO_2 in mm Hg			CO_2 in mEq			
NAME	SEX	AGE	B	D	A	B	D	A	B	D	A	ILLNESS
F J	M	70	7.59	7.52	7.58	41.81	42.65	49.33	29.0	29.0	29.0	Inguinal hernia
J W	M	17	7.55	7.51	7.51	40.28	29.60	43.80	28.0	27.0	24.0	Appendicitis
I H	M	49	7.55	7.52	7.50	45.21	43.98	41.95	28.0	28.0	29.0	Fistula in ano
J H	M	39	7.50	7.49	7.51	63.26	52.62	53.44	40.0	37.0	37.0	Stab wound
M H	M	65	7.51	7.52	7.49	46.86	46.09	49.33	30.0	31.0	30.0	Hemorrhoids
AVERAGES			7.54	7.51	7.52	47.48	42.99	47.57	31.0	30.4	29.4	

B—Before D—During A—After

potassium, intracellular potassium, intracellular potassium concentration in intracellular water, residual sodium,† and residual sodium concentration in intracellular water.

It should be emphasized that the methods used in this combined procedure are not new.[1,2,3,4] When combined in this procedure, minor modifications are necessary.

The separation of the radioactive isotopes involved is accomplished easily by serial administration, interval counting, and by a chemical separation of potassium.

MATERIALS

Ten normal subjects and 60 hospitalized patients have been studied during the past year by this simultaneous combined method of determination of body composition. The 60 hospitalized patients included individuals with cardiac disease, renal disease, malnutrition, inflammatory processes, carcinoma, and other forms of chronic wasting illness.

RESULTS

Normal Values. The values in the normal group are in good agreement and are in the range of those previously reported. Table 1 lists mean

Table 1. Normal Values for a Hypothetical Male of Age 60 Years and Weight 70 Kg

MEASUREMENT	ABSOLUTE VALUE	RELATIVE VALUE AS % BODY WEIGHT OR mEq/kg
Weight	70 kg	— —
Hct, Large Vessel	44%	— —
Hct, Whole Body	40%	— —
Plasma Volume	3150 cc	4.5%
Red Blood Cell Volume	2100 cc	3.0%
Blood Volume	5250 cc	7.5%
Total Body Water	39.8 L	56.9%
Extracellular Water	16.4 L	23.4%
Intracellular Water	23.4 L	33.4%
Total Exchangeable Chloride	2030 mEq	29 mEq/kg
Plasma Chloride Concentration	105 mEq/L	—
CO_2 Combining Power	27 mEq/L	
Plasma Sodium Concentration	140 mEq/L	
Total Exchangeable Sodium	2870 mEq	41 mEq/kg
Extracellular Sodium	2340 mEq	33.4 mEq/kg
Residual Sodium	530 mEq	7.6 mEq/kg
Residual Sodium Concentration in Intracellular Water	23 mEq/L	
Total Exchangeable Potassium	3300 mEq	47 mEq/kg
Extracellular Potassium	67 mEq	1 mEq/kg
Intracellular Potassium	3230 mEq	46 mEq/kg
Intracellular Potassium Concentration in Intracellular Water	138 mEq/L	
Plasma Potassium Concentration	4.0 mEq/L	
Serum Osmolarity	285 mOsm/L	

†The term "residual sodium" is used to denote that portion of the total exchangeable sodium not accounted for in the extracellular fluid and presumably residing in muscle cells and bone.

an average of 13 1 per cent during infusion of the sodium lactate in 5 subjects and decreased an average of 17 2 per cent during the infusion of ammonium chloride in 5 subjects The CO_2 increased an average of 17 3 per cent during the sodium lactate infusion and decreased an average of 17 3 per cent during the ammonium chloride infusion An increase in the blood pH of 0 06 followed infusion of sodium lactate and a decrease of 0 06 followed the infusion of ammonium chloride Hypertonic saline had little effect upon these values

The participation of the respiratory bicarbonate buffer system in neutralizing infused acid or alkali is again demonstrated Respiratory compensation did occur following the infusion of both acid and alkali

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THE EVALUATION OF BODY COMPOSITION IN SURGICAL DISEASE PROCESSES UTILIZING A METHOD FOR THE SIMULTANEOUS DETERMINATION OF RED BLOOD CELL VOLUME PLASMA VOLUME BLOOD VOLUME TOTAL BODY WATER EXTRACELLULAR WATER AND TOTAL EXCHANGEABLE CHLORIDE SODIUM AND POTASSIUM*

JAMES D MCMURREY JOHN M DAVIS ELDON A BOLING AND FRANCIS D MOORE

Various aspects of body composition have been extensively studied in the past but most have been determinations of 1 2 and rarely 3 constituents at the same time[1, 2, 3, 4] Accurate correlation of body constituents in disease states has been heretofore largely impossible because of the time interval between measurements

METHOD

In this laboratory a method has been developed for the determination over a period of 48 hours of red blood cell volume with Cr^{51} plasma volume with T 1824 total body water with D O extracellular water volume with Br^{82} total exchangeable chloride with Br^{82} total exchangeable sodium with Na^{24} and total exchangeable potassium with K^{42} Additional determinations of serum osmolarity plasma protein concentration carbon dioxide combining power and plasma concentrations of chloride sodium and potassium were done

These directly determined measurements of body composition also permit calculation of intracellular water volume extracellular sodium extracellular

---

*From the Department of Surgery Harvard Medical School and the Surgical Service and Laboratories of the Peter Bent Brigham Hospital Boston Massachusetts This work was sponsored in part by the Committee on Metabolism in Trauma Commission on Liver Diseases Armed Forces Epidemiological Board and supported by the Surgeon General Department of the Army through a contract (DA-49 007 MD 472) with Harvard University and in part by the U S Atomic Energy Commission through a grant to the Peter Bent Brigham Hospital (AT (30 1)—733)

and prolonged convalescence due to the effects of massive trauma These patients have been grouped with regard only to the degree of weight loss and sex Representative findings in cachexia not associated with heart disease are seen in Table 2 which lists the mean values obtained from study of 10 male patients with severe chronic wasting illness Mean weight loss was 21 5 per cent of the weight in health In this table the results have been expressed as a percent of normal based on the observed weight and as a percent of normal based on the weight in health

DISCUSSION

To quantitate the changes which take place during the process of a chronic illness from health to disease it would be ideal to do serial measurements of body composition during the disease process and to have a pre depletion baseline The latter obviously is not possible in most instances The findings in disease have therefore been compared to those of a hypothetical normal individual of the same sex and weight as the patient before onset of illness (weight in health)

The data presented for the group of cachectic patients show a striking correlation of the changes which take place in extracellular water volume total exchangeable chloride total exchangeable sodium and plasma volume This correlation might be predicted and is not surprising for sodium and chloride are chiefly extracellular electrolytes and plasma volume is a compartmentalized portion of the extracellular fluid volume Those measurements of the changes which relate to intracellular substance intracellular water volume total exchangeable potassium and red cell volume show a similar correlation The total body water and blood volume also show a close correlation

These correlations hold for the two standards of reference that is observed weight and weight in health If the standard of reference be observed weight the extracellular water volume total exchangeable chloride total exchangeable sodium and plasma volume are markedly increased the total body water and blood volume are normal and the intracellular water volume total exchangeable potassium and red blood cell volume are markedly decreased Relative to weight in health measurements of the extracellular substances are normal measurements of the total body water and blood volume are low and measurements of the intracellular substances are markedly decreased

This can only mean that as depletion take place the extracellular constituents (extracellular water sodium chloride and plasma volume) remain essentially unaltered while the major change is of the intracellular substances (intracellular water potassium and red blood cells) Total body water since it is composed both of extracellular water and intracellular water strikes a balance midway between the extracellular stability and the intracellular decrement The blood volume also strikes a balance between plasma volume stability and red blood cell depletion

Chronic cardiac patients are cachectic and in this regard are similar to cachectic patients without heart disease There are differences of some note however First patients with non cardiac cachexia have blood volumes low for weight in health whereas cardiac patients have a larger blood volume than is expected in health *Non cardiac cachexia* is associated

normal values for a hypothetical male of age 60 years and weight 70 kg *Space does not permit enumeration of the values for the female which vary from those of the male chiefly because of a difference in fat and lean tissue mass*

Body Composition in Chronic Wasting Illness Interpretation of the values from individual cases may be misleading because of the wide range of normal variation Therefore for purposes of analysis the mean values obtained from a group of patients having the same pattern of compositional defect have been related to the mean figures for a group of normal individuals of comparable sex and age Early in the course of this investigation it became apparent that there was a similar pattern of body compositional alteration in those individuals having chronic wasting illnesses of various causes excluding that associated with cardiac disease The group of patients in this category are representative of a variety of clinical conditions *including chronic sepsis malignancy malnutrition intestinal obstruction*

Table 2 Mean Values of 10 Males with Severe Cachexia Not Associated with Cardiac Disease Average Age 63 years

MEASUREMENT	ABSOLUTE VALUE	RELATIVE VALUE % BODY WEIGHT OR mEq /kg	% NORMAL BASED ON OBSERVED WT	% NORMAL BASED ON WT IN HEALTH
Weight	55 kg	–	–	78.5
Hct Large Vessel	37 6%	–	85	85
Hct Whole Body	32.5%		81	81
Plasma Volume	2970 cc	5 4 %	120	91
R B C Volume	1430 cc	2.6 %	87	67
Blood Volume	4400 cc	8 0 %	107	83
Total Body Water	30.3 L	55 1 %	97	76
Extracellular Water	16 6 L	30 1 %	129	101
Intracellular Water	13 7 L	24 9 %	75	58
Total Exchangeable Chloride	1950 mEq	35.5 mEq /kg	122	96
Plasma Chloride Concentration	102 mEq /l		97	97
CO_2	25 mEq /L		100	100
Plasma Sodium Concentration	133 mEq /l		95	95
Total Exchangeable Sodium	2720 mEq	49.5 mEq /kg	120	95
Extracellular Sodium	2250 mEq	40 9 mEq /kg	120	96
Residual Sodium	470 mEq	8.6 mEq /kg	113	88
Residual Sodium Concentration in Intracellular Water	34.3 mEq /l		149	149
Total Exchangeable Potassium	1990 mEq	[illegible]1 mEq /kg	77	60
Extracellular Potassium	80 mEq	1 4 mEq /kg	150	91
Intracellular Potassium	1910 mEq	34 7 mEq /kg	75	59
Intracellular Potassium Concentration in Intracellular Water	139 mEq /l		101	101
Plasma Potassium Concentration	4 3 mEq /l		108	108
Serum Osmolarity	270 mOsM/L		95	95

This report deals with (a) certain additional experimental studies on intravenous fat emulsions in animals and (b) a clinical evaluation of three commercial preparations of intravenous fat emulsions

EXPERIMENTAL STUDIES

This phase of the study was an attempt to evaluate the assimilation of fat introduced intravenously in the dog For this purpose dogs were fed a fatty meal of [illegible] gm/kg peanut oil containing 100 microcuries of I^{131} labelled *glycerol trioleate*† The thoracic duct of the animal was then cannulated and a quantity of radioactive thoracic duct fat was collected This material was then injected intravenously into another dog which had been given 99 gm potassium iodide to saturate the thyroid Radioanalysis of periodic blood samples was then made in a scintillation well counter using 2 cc of venous blood At the end of 24 hours the animal was sacrificed and radioanalysis made on selected organ samples Fig 1 shows the calculated per cent of radioactivity present in the blood of 2

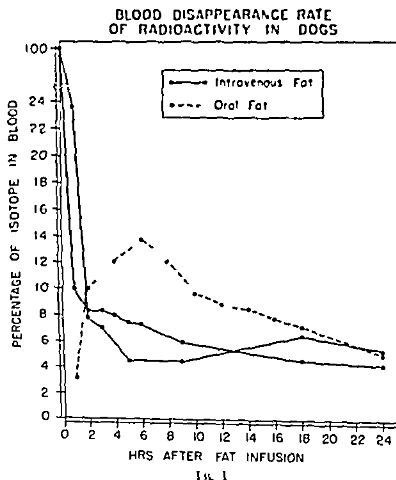


Fig 1

dogs at varying intervals up to 24 hours after introduction of a measured amount of radioactive labelled fat intravenously A comparative curve is shown in which a larger quantity (100 microcuries) of radioactive fat was administered by stomach tube It is seen that approximately 90 per cent of the radioactivity disappears from the blood in 2 hours and at the end

†Obtained from Abbott Laboratories Chicago Illinois under A E C authorization

with a relatively low red blood cell volume and a relatively high plasma volume Red cell volume and plasma volume are both high in cardiac cachexia In non cardiac cachexia total exchangeable chloride, total exchangeable sodium, extracellular water and plasma volume are maintained at near normal levels for the usual weight (high for weight in illness) In cardiac cachexia total exchangeable chloride total exchangeable sodium extracellular water and plasma volume are increased even above normal levels for weight in health

SUMMARY

1 Body composition studies were obtained in 10 normal and 60 patients with varying disease processes

2 Values considered normal for a male of 60 years of age are presented

3 Representative values are given for a group of patients with severe cachexia

4 There was a similarity of findings in those patients having chronic wasting illness of whatever cause except cardiac disease

REFERENCES

1 James A H Brooks L Edelman I S Olney J M and Moore F D Body sodium and potassium I Simultaneous measurement of exchangeable sodium and potassium in man by isotope dilution Metabolism *3* 313 1954

2 Moore F D Haley H B Bering E A Jr Brooks L and Edelman I S Further observations on total body water II Changes of body composition in disease Surg Gyn Obst *95* 155 1952

3 Read R C Studies of red cell volume and turnover using radiochromium N England J Med *250* 1021 1954

4 Von Porat B T D Blood volume determination with Evans blue dye method Acta med scand Suppl *140* 256 1951

STUDIES ON INTRAVENOUS FAT EMULSIONS*

WILLIAM W SHINGLETON W G ANLYAN, MOHAMMED EL HADI SALEM AND AARON P SANDERS

Since all caloric solutions currently in use are inadequate to supply the daily caloric needs of patients the need for a stable non toxic fat emulsion for parenteral nutrition is generally recognized This need for such a preparation of fat was recognized many years ago In fact fat was injected intravenously long before carbohydrate and protein Thus Courten[1] in 1679 attempted to give fat intravenously The modern era in intravenous fat emulsions was inaugurated in 1935 by Holt *et al*[2] who by homogenization and use of lecithin for emulsification were able to prepare emulsions considered safe for intravenous use Since 1915 numerous studies and reports have been made notably by Stare Shafiroff Meng and Freeman Grossman, and Cannon

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Table 3 Types of Reactions in 113 Intravenous Fat Infusions

CHILL	10
BACK PAIN	6
FEVER	5
NAUSEA & VOMITING	2
CHEST PAIN	2
GENERALIZED CRAMP	2[illegible]
TOTAL	2[illegible]

of frequency the type of reactions encountered. Two patients who died from their underlying disease received intravenous fat several days prior to death. Autopsies were obtained on both of these patients. One patient, a 56 year old male with irresectable carcinoma of the esophagus who had undergone prolonged starvation and dehydration, received 2100 cc. of olive oil emulsion during a 24 hour period 2 days prior to death. Multiple fat emboli were demonstrated at autopsy in the heart, brain and lungs. The other patient, a 65 year old female, received 1000 cc. of the sesame oil emulsion and died 12 hours later from a massive gastrointestinal hemorrhage secondary to lymphosarcoma of the stomach. Autopsy examination revealed no evidence of fat emboli in any of the organs.

Selected Hematologic Studies. Coagulation time of the blood was studied in 10 patients with no significant change observed before or after intravenous fat emulsions. The largest variation found was an increase from 8 minutes to 15 minutes in one patient. No significant change in bleeding time was encountered in 5 patients. Platelet counts prior to and after 600 cc. of intravenous fat emulsion showed no outstanding alteration in 11 patients. *Studies on red cell fragility in 6 patients* before and after 600 cc. of intravenous fat revealed no abnormalities.

Nitrogen Balance Studies. Nitrogen balance studies were carried out in 3 patients undergoing subtotal gastric resection for duodenal ulcer. Ni

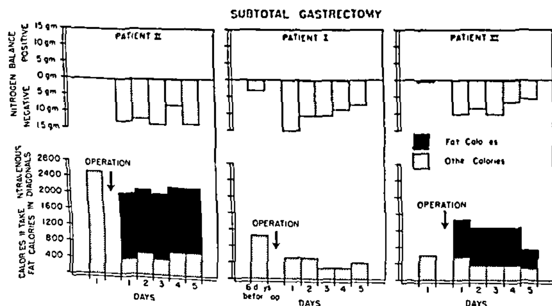


Fig 2

of 24 hours approximately 5 per cent remains Table 1 lists the organ analysis of 2 dogs which received intravenous radioactive fat emulsion and 1 dog which received the larger amount of material by stomach tube The organs showing the largest content of radioactive fat after 24 hours were liver lungs and spleen

Table 1 24 Hour Residual of Radioactivity Found on Organ Analysis After Intravenous Fat Infusion

	DOG I*	DOG II†	DOG III‡
LIVER	3 1	3 1	1 5
LUNG	38	.22	13
SPLEEN	92	65	91
KIDNEY	137	11	34
THYROID	32	06	.06

*½ gm fat/kg containing 100 microcuries I^{131} given by mouth
†7 cc /kg sesame oil emulsion containing 3 microcuries I^{131}
‡7 cc /kg sesame oil emulsion containing 12 microcuries I^{131}

CLINICAL STUDIES

This phase of the report deals with (1) the frequency and types of reactions observed in patients receiving intravenous fat emulsions (2) selected hematologic studies on patients receiving intravenous fat emulsion and (3) the effect of intravenous fat emulsion on the negative nitrogen balance occurring immediately following major surgical trauma and during prolonged parenteral feeding in surgical patients with deficient caloric intake

Reactions Thirty eight patients were given a total of 113 infusions of intravenous fat emulsions Three commercial fat preparations were used in the study* each containing a different oil as the source of fat but each using lecithin as the emulsifying agent No medication was given prior to or during the administration of the emulsion Patients with a wide variety of diseases were used as subjects however the majority were patients in various stages of neoplastic disease Table 2 lists the reaction rates encountered with the three emulsions used Table 3 lists in order

Table 2 Reaction Rates in 114 Intravenous Fat Infusions

OIL	NO PATIENTS	NO INFUSIONS	NO REACTIONS	% REACTIONS
SESAME*	20	74	12	16
OLIVE†	7	29	9	31
COTTONSEED‡	11	11	4	36
TOTALS	38	114	25	21

* Intravenous Fat Emulsion Don Baxter Inc 500 cc 540 cal
†"Lipomul Upjohn Co 600 cc 810 cal
‡ Lipomul Upjohn Co 600 cc 810 cal

*Intravenous Fat Emulsion (sesame oil 10%) Don Baxter Inc Glendale California Lipomul (olive oil 15%) Upjohn Co Kalamazoo Michigan Lipomul (cottonseed oil 15%) Upjohn Co Kalamazoo Michigan

tion to prevent particles from coalescing, and sterilization appears to have been achieved in certain commercial preparations available for investigational use. Although no severe acute or chronic toxicity has been encountered, there remains the problem of mild systemic reactions occurring in 15 to 25 per cent of patients given intravenous fat emulsions. A wide variety of reactions have been reported, including chills, fever, nausea and vomiting, back pain, chest pain, hypo- and hypertension, and urticaria. The cause of these reactions is not known.

No severe reactions were encountered in the present study. The most common reaction seen was a mild chill followed by a temperature rise of .5 to 1° C. The rate of administration appears important, and often when reactions occurred, cessation of the infusion for 5 to 10 minutes relieved the patient's symptoms and permitted continuation of the infusion without further reaction.

In view of the autopsy findings of multiple emboli in one of the patients reported here who received 2100 cc. of intravenous fat emulsion during a 24 hour period, a definite quantitative limit should be placed on the amount of the material given during any single day. A limit of 1000 cc. daily would appear desirable.

The type of fat used in preparation of the intravenous emulsion appears to be related to reaction rate. In this study sesame oil emulsion gave a definitely lower reaction rate than did preparations of olive oil or cottonseed oil.

Further studies are under way to evaluate the effectiveness in prevention of reactions of an antihistaminic drug and/or heparin given just prior to the fat infusion.

Finally, it appears that the most effective and desirable indication for intravenous fat emulsions in surgical patients is in those patients where underlying disease or surgical complications preclude an adequate oral intake of calories for a prolonged period.

SUMMARY

One hundred fourteen intravenous fat infusions consisting of 3 different preparations (cottonseed oil, olive oil and sesame oil) have been given to patients for evaluation of reaction rate, bleeding and clotting times, changes in red cell fragility and platelet count. In addition nitrogen balance studies have been carried out in certain selected postoperative patients to test the effect of intravenous fat on the negative nitrogen balance occurring in the early postoperative period and the later negative balance in patients with surgical complications leading to prolonged limitation of caloric intake. A radioactive labelled fat (glycerol trioleate I^{131}) has been used in dogs to determine the blood disappearance rate of radioactivity and the site of organ uptake.

Results of these studies reveal a reaction rate of 16 to 36 per cent with the various preparations. Intravenous fat appears to be effective in decreasing the negative nitrogen balance occurring during prolonged inadequate caloric intake but not effective in doing this in the early postoperative period. No effect on red cell fragility, blood coagulation or platelet count was detected.

trogen determinations were made on aliquots of 24 hour collections of urine gastric aspiration and feces using the micro-Kjeldahl method Two of these patients received 1000 cc of fat emulsion intravenously daily in the early postoperative period during this phase of parenteral therapy Figure 2 shows the result of this study and indicates that intravenous fat given early in the postoperative period does not influence to any extent the negative nitrogen balance occurring after major surgical trauma Two additional patients undergoing prolonged parenteral feeding because of paralytic ileus following peritonitis were studied The study was begun on the fifth day following laparotomy and total intake and output of nitrogen was measured Both of these patients showed a reduction in negative nitrogen balance following the addition of calories in the form of intravenous fat emulsion (See Fig 3)

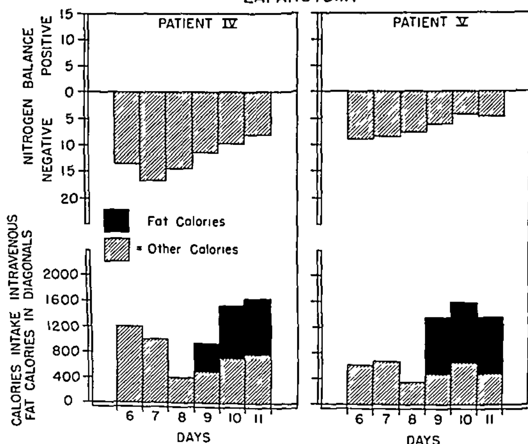


Fig 3

DISCUSSION

Previous studies have established in a variety of ways the fact that fat given intravenously is metabolized[3,4,5,6,7] Also rather extensive studies on erythrocyte sedimentation rate hematocrit serum Na K and chloride levels serum bilirubin (direct and indirect) thymol turbidity cephalin flocculation alkaline phosphatase cholesterol cholesterol esters and serum albumin and globulin have disclosed no deviations from normal in patients receiving intravenous fat emulsion[8] The problem of globule size stabiliza

sources of fat suitable for intravenous use. Other than sporadic experiments with lard, butter or thoracic duct chyle, no effort has been made to adapt animal fats to intravenous use. It was thought that animal body fat might furnish a more physiological source of neutral fat for parenteral nutrition in man.

Beef fat was selected as the basis of our preparation. Beef fat, in the form of suet, was obtained from the retail butcher and ground at the store in an electric grinder. The ground suet was then dissolved in petroleum ether (boiling point 30 C. to 60 C.) in a ratio of 5 parts ether to 1 part suet. All soluble material was dissolved in the ether by agitation with a motor-driven rotary blade. The temperature was maintained at approximately 65 C. in a water bath. On standing for a few minutes at room temperature the insoluble fibers and gristle settled out of the ether solution and the supernatant fluid was then filtered through laboratory towels. The ether was evaporated by blowing compressed nitrogen through the solution leaving a yellow fat, solid at room temperature. We shall refer to this fat as *whole beef fat*.

A 15 per cent emulsion of whole beef fat was prepared in 5 per cent dextrose with 1 per cent Asolectin, a soy bean phosphatide, as the emulsifier, by means of high pressure homogenization in the Hydropulse H B–2S homogenizer. The emulsion was placed in standard infusion bottles and was autoclaved in these closed containers as the final step in its preparation. Ninety per cent of the particles of this emulsion after autoclaving were 1 μ or less in size.

When this whole beef fat emulsion, freshly prepared, was infused intravenously into a series of dogs the results were variable and unpredictable. The same batch of fat was received by some dogs without reaction. In other animals it caused salivation, vomiting and even death. Small amounts were well tolerated in all animals. Because of the variability of these

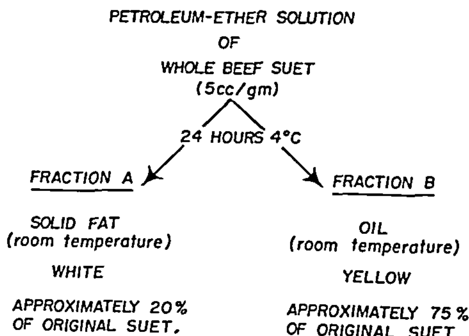


Fig 2 Fractionation of whole beef fat

REFERENCES

1 Courten W Proc R Soc M Lond 27 495 1710 1712 1933 34
2 Holt L E Jr Tidwell H C and Scott T F M The intravenous administration of fat J Pediat St Louis 6 151 (Feb) 1935
3 Waddell W R Geyer R P Grillo H C and Stare F J The treatment of malnutrition with particular reference to the surgical patient Am J Surg 88 698 (Nov) 1954
4 Meng H C and Freeman S Experimental studies on the intravenous injection of a fat emulsion into dogs J Laborat Clin M 33 689 1948
5 McKibben J M Ferry R M Jr and Stare F J Parenteral nutrition II The utilization of emulsified fat given intravenously J Clin Invest 25 679 1946
6 Gordon H H and Levine S Z Respiratory metabolism in infancy and childhood XVI Effect of intravenous infusions of fat on the energy exchange of infants Am J Dis Child 50 894 1935
7 Geyer R P Chipman J and Stare F J Oxidation *in vivo* of emulsified radioactive trilaurin administered intravenously J Biol Chem 176 1469 1948
8 Personal communication from Dr Paul Jordan through Don Baxter Inc

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THE DEVELOPMENT OF A PREPARATION OF PURIFIED ANIMAL FAT SUITABLE FOR INTRAVENOUS USE*

MARY ANN PAYNE NATHAN BROT CLAUDE CHOLETTE AND JOHN M BEAL

Previous studies on fat emulsions have focused attention primarily on fats of vegetable origin coconut corn cottonseed olive and linseed oil as

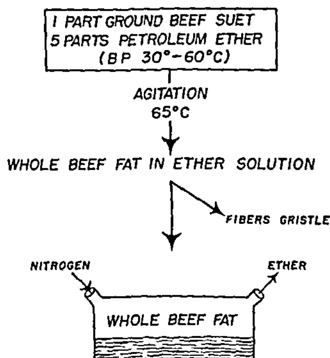


Fig 1 Preparation of whole beef fat

*From the Departments of Surgery and Medicine of the New York Hospital Cornell Medical Center This investigation was supported in part by research grant A 181 from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health Public Health Service and in part by The Astor Foundation

Both *Fraction A* and *Fraction B* were prepared in 15 per cent emulsions in the manner previously described and were infused separately into 2 series of dogs. The results were strikingly different with the 2 fractions. Table 2 shows the results of infusions with *Fraction A* in a series of 12 dogs. The fraction was highly lethal. Small amounts, as little as 5 cc, caused immediate death in 10 of the 12 dogs. The 2 surviving dogs recovered only after severe reactions. *Fraction B*, on the other hand, was given consecutively to 25 dogs in amounts of 200 cc. to 550 cc. per infusion or 3 to 7.5 gm. per kg. of body weight. Multiple infusions in 3 dogs were also well tolerated. One additional dog received 7 infusions or a total of 293 gm. of fat over a period of 2 months. Four monkeys also received *Fraction B* intravenously in amounts of 2 gm. per kg. No reaction occurred to any of these infusions.

The high toxicity of the A fraction was of great interest to us. The cause of death in these animals was not apparent by gross examination at the time of autopsy. The lungs of all dogs were clear. The peritoneal cavity did not show any gross lesions. There were no petechiae. Post-mortem centrifuged blood revealed lipemic serum but hemolysis was not present. However, histologic studies demonstrated the presence of fat emboli in the lung and in the small vessels of the heart. Sections of the liver and of the brain failed to show significant quantities of fat.

Difference in toxicity in various fractions of coconut oil were described by Shafiroff[1] *et al.* and attributed to differences in molecular weight of the fatty acids of the triglycerides. The fraction containing the short-chain fatty acids caused hemolysis while the long-chain fraction was relatively non-toxic. Since the neutral fat stored by animals as body fat consists almost entirely of longer chain fatty acids[2] one does not encounter this difficulty in working with animal fat emulsion. The difference in the toxic and non-toxic beef fractions appears to be primarily one of the degree of saturation of the fatty acids. Both chain length and degree of saturation of fatty acids, therefore, appear to be significant in determining the toxicity of fat emulsions. It is to be hoped that more detailed studies of toxic and non-toxic fat fractions may reveal other factors which are important in causing the commonly observed reactions to intravenous fat infusions.

REFERENCES

1. Shafiroff, B. G. P., Mulholland, J. H., and Baron, H. C.: Intravenous infusions into human subjects of fractionated coconut oil emulsions. Proc. Soc. Exp. Biol., N. Y. *79*: 721-723, 1952.

2. Hilditch, T. P., and Longenecker, H. E.: A further study of the component acids of ox depot fat with special reference to certain minor constituents. Biochem. J., Lond. *31*: 1805-1819, 1937.

results an attempt was made to purify the beef fat The original ether solution of whole beef fat was placed in the refrigerator at 1°C for 24 hours A snowy white precipitate was obtained approximately 20 per cent by weight of the original suet This solid white fat was removed by fil tration from the fat solution and was dried in the air This fraction will be designated as *Fraction A* After *Fraction A* had been removed the ether was blown off from the filtrate under compressed nitrogen A clear yellow oil remained which will be called *Fraction B*

The chemical analysis of the beef fat and its fractions is presented in Table I The whole beef fat does not contain any protein phospholipid or cholesterol Furthermore the absence of fat soluble vitamins and sterols is indicated by the lack of any unsaponifiable material The saponification values are fairly uniform for the 3 fractions indicating uniform chain lengths throughout *Fraction A* has a high melting point and a low iodine number which indicates a high degree of saturation whereas the oil *Fraction B*, has a lower melting point and a higher iodine number which indicates a higher degree of unsaturation

Table 1 Chemical Analysis—Whole Beef Fat

		FRACTION A	FRACTION B
Melting Point	47 8 C 48 1 C	53 9 C 55 0 C	25 0 C 27 8 C
Iodine Number	40 0 41.2	1 3 19 7	46 6 54 6
Saponification Number	191 196	202 206	186 190
Unsaponifiable Material	Absent		
Protein	Absent		
Phospholipid	Absent		
Cholesterol	Absent		

Table 2 Results of Intravenous Infusion of 15 per cent Emulsion of Fraction A

TOTAL FAT INJECTED (GM)	NUMBER OF DOGS	REACTIONS	DEATHS
0 75 1.5	3	1	2
3 0 4 5	3	1	2
6 0 11 3	3	0	3
15 0 16 5	3	0	3
	12	2	10

Table 3 Results of Intravenous Infusion of 15 per cent Emulsion of Fraction B

QUANTITY GM/KG	NUMBER OF DOGS	REACTIONS	DEATHS
3 3.5	10	0	0
4 4.5	11	0	0
5 6	2	0	0
7 7.5	2	0	0
	25	0	0

fat emboli blood samples from 20 convalescent patients were examined before and 4 hours following the intramuscular administration of 25 mg. of ACTH. Blood samples from 6 medical students were similarly examined following the intramuscular injection of an equal volume of isotonic sodium chloride solution.

In all instances blood samples were examined for fat globules according to the method of Peltier[1] as outlined below.

One-half ml. of a 1:1000 aqueous solution of the flurochrome fat stain phosphin 3R*, was added to a 3 to 5 ml. specimen of oxalated venous blood. The tubes were centrifuged for 20 minutes at 2500 rpm. A series of 8 to 12 drops were transferred by an applicator stick from the meniscus of the supernatant fluid to a glass slide and allowed to dry. Fat globules were identified by ultraviolet microscopy utilizing an ordinary clinical microscope modified as follows.

The usual substage condenser, microscope slides, objective and eyepiece were employed but an ultraviolet light source (G. E. No. 31-31-53) was used in a "tower type" microscope illuminator. Much of the heat from the ultraviolet light source was eliminated by interposing a violet filter (Bausch and Lomb No. 31-31-51) between the light source and an aluminum "clip-on" mirror which slides over the regular mirror. An eyepiece cap filter (Bausch and Lomb No. 31-51-25), was placed over the regular eyepiece to protect the observer's eye from the ultraviolet rays.

Fat emboli appeared under the 16 ml. objective of the ultraviolet microscope as yellow or orange spherical fluorescent particles ranging from 10 to 22 micra in diameter as measured by an eyepiece micrometer (Figure 1). Globules of a similar size but of a waxy appearance and a white to green color have been shown by Peltier[10] to be pollen granules.

RESULTS

Controls. In the unoperated fasting control group 1 individual of the 30 tested was positive for fat globulemia. Of the 10 convalescent patients examined before and after a fatty meal 1 patient exhibited fat globules in the peripheral blood following the meal.



Fig. 1

*Obtained from Pfaltz and Bauer Co., New York City, New York.

THE INCIDENCE OF FAT GLOBULEMIA FOLLOWING SOFT TISSUE AND ORTHOPEDIC OPERATIONS*

William A. Bryans and Ben Eiseman

In general, there are two theories as to the pathogenesis of fat embolism: one maintaining that fat particles enter the venous sinuses from the marrow of long bones at the time of fracture[1,7,8] while others feel that trauma or stress cause a clumping of the circulating chylomicrons or an alteration of the normal fat emulsion in the blood.[2,3,4,5,6]

Recently Peltier[1] described a new method for detecting fat emboli in peripheral blood utilizing ultra violet microscopy and reported the appearance of fat globules in the peripheral blood in a large percentage of individuals following orthopedic procedures. He found a much lower incidence of fat globules in those patients undergoing surgery without trauma to long bones but analysis of these cases reveals that many of the soft tissue procedures were relatively minor surgical operations compared to the major orthopedic procedures upon which his study was based.

The purpose of this study is to compare the incidence of fat globulemia in the peripheral blood of patients undergoing orthopedic procedures with those experiencing soft tissue surgery of similar magnitude.

MATERIALS AND METHODS

Clinical material consisted of 130 patients undergoing surgery at the Denver Veterans Administration Hospital, General Rose Memorial Hospital and The Colorado General Hospital. These patients were divided into orthopedic and soft tissue surgery groups and these groups subdivided somewhat arbitrarily into patients undergoing major and minor operative procedures. Fasting preoperative blood samples of 3 to 5 ml. were drawn into oxalate tubes 1 to 4 hours prior to operation and a similar sample was obtained 30 to 60 minutes following return of the patient to the recovery room.

A control group consisting of 30 young healthy blood donors was similarly examined for the presence of fat globules in fasting blood samples. Blood samples from 10 additional convalescent patients from the orthopedic wards were examined before and 3 hours following a fatty meal, to determine whether or not an alimentary lipemia alone would produce circulating fat globules.

The blood of 5 fasting dogs under sodium pentobarbital anesthesia was examined for fat globules both before and after fractures of all four extremities. A pre-fracture blood sample was obtained, and 30 minutes following fracture of each extremity a blood sample was obtained from the external jugular vein. A transverse fracture was produced by a single mallet blow on the extremity which was immobilized between 2 lead blocks. Thirty minutes after fracture of the fourth extremity a blood sample was taken from the right atrium.

In order to evaluate the role of "stress" in the appearance of circulating

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DISCUSSION

Our findings are in agreement with those of Peltier[1] as to the high incidence of fat globulemia in the peripheral blood following major orthopedic operations. The comparable incidence of fat globulemia following soft tissue operations of similar magnitude suggests that the degree of operative trauma rather than manipulation of bone is of major importance in the appearance of these fat particles. Minor orthopedic and soft tissue procedures revealed essentially the same low incidence of postoperative fat globulemia.

The appearance of fat globules in 21 preoperative cases deserves special comment. Four of these preoperative positives were fracture cases and preoperative manipulation may have been responsible for this finding.[3] Three other patients undergoing amputations of gangrenous extremities were positive for fat globulemia preoperatively and revealed a decrease in the number of globules following amputation. Two cases of chronic osteomyelitis and 1 case of chondrosarcoma were also positive preoperatively. We have no logical explanation for the appearance of fat particles in the blood of the other 11 patients prior to surgery. It was found that when these cases were included with the negative preoperative patients the difference in the final results was not statistically significant.

Since stress has been suggested as a factor in the pathogenesis of fat embolism, a group of patients was tested by a challenge dose of ACTH. Fat particles were not seen in an appreciable number of these individuals.

The exact origin of the embolic fat remains unsettled. Peltier has shown that the fat globules seen in the peripheral blood are actually embolic to the lung, brain and kidney in the dog.[1] The distribution of the fat positive blood samples following multiple fractures in dogs suggests that the finding of fat emboli is not dependent upon the amount of bone marrow fat released at the time of fracture. Although our method was admittedly not quantitative, the number of globules seen in peripheral blood samples was not related to the number of extremities previously fractured. These results place considerable question upon the marrow theory of origin of embolic fat and support the hypothesis that fat emboli in the peripheral blood may result as a response to stress, or from manipulation of soft tissue regardless of bone involvement.

Evidence that clumping of the chylomicrons of the blood can be responsible for fat embolism is wanting. Whiteley showed that there was no greater degree of pulmonary fat embolism after muscle ischemia in the fat fed animal than in the fasting animal.[9] Others have suggested that during states of stress, high concentrations of lipids and of lipase may liberate excessive quantities of fatty acids and other products capable of causing an alteration of the formed elements of the blood which would favor the development of emboli.[6]

SUMMARY AND CONCLUSIONS

1. The incidence of fat globulemia in the peripheral blood of a series of patients undergoing soft tissue and orthopedic operative procedures is reported and correlated with the degree of operative trauma involved rather than with the presence or absence of long bone manipulation.

Soft Tissue Operations Blood samples from 67 patients were examined before and after soft tissue operations of various types (Table 1) The group consisted of 51 major and 16 minor operative procedures Of the 51 patients in the major surgery group 24 or 47 0 per cent, revealed fat globulemia postoperatively Of the 16 patients undergoing minor operative procedures only 3 or 18 8 per cent were positive for fat globules For reasons not clear to us 12 patients awaiting soft tissue operative procedures had positive preoperative examinations for fat globules

Orthopedic Operations. Blood samples from 37 patients undergoing various types of orthopedic procedures were examined (Table 1) The group consisted of 15 major and 22 minor operative procedures Of the 15 patients in the major surgery group 10 or 66 6 per cent revealed fat globules post operatively In the minor surgery group 5 of the 22 patients or 22 7 per cent were positive for the presence of fat postoperatively In this group there were also 12 preoperative blood samples positive for fat globules

The 24 patients equally divided between soft tissue and the orthopedic group with positive preoperative specimens were not included in the data presented There was no correlation of the presence of fat particles with age in either group

ACTH Group Only 2 of the 20 convalescent patients with a negative pre injection examination who were challenged with 25 mg of ACTH exhibited fat globules in the peripheral blood Two additional patients with a small number of globules in the peripheral blood prior to ACTH injection showed an apparent increase following adrenal stimulation One of the 6 medical students was positive for fat globules in the peripheral blood both before and after an intramuscular injection of isotonic saline solution

Experimental Fractures in Dogs Table 2 summarizes the results following experimental fracture of the femur of anesthetized dogs In each case circulating fat globules were observed at least upon one examination but in 9 of the 20 examinations no fat globules were found If these globules arose from the fracture site one would expect a higher incidence of globulemia

Table 1 Incidence of Fat Globulemia in Postoperative Blood Samples

TYPE OF SURGERY	MAJOR SURGERY	%	MINOR SURGERY	%
Orthopedic	10/15	66 6	5/22	22 7
Soft tissue	24/51	47 0	3/16	18.8

Table 2 Incidence of Fat Globulemia in Dogs Following Fractures of All Four Extremities

DOG NO	PRE FRACT	1ST FRAC	2ND FRAC	3RD FRAC	4TH FRAC
1	0	+	+	+	+
2	+	0	+	0	+
3	0	+	+	0	0
4	0	+	+	0	0
5	0	0	0	+	0

in each. The individuals in each group received either 500 or 1000 cc of the expander and were followed at intervals for 24 hours. The average curve for each expander group was used for the analysis.

The method of graphic analysis is that described by Solomon and by Cohn and Prues. It consists in plotting the curve of disappearance (per cent of amount given versus time) on semi-logarithmic paper and then subtracting the extrapolated terminal straight line segment from the curve. If the resulting values also give a curve the process is repeated until a straight line results. If the first subtraction gives a straight line the process is finished. The compound curves are thus resolved into a series of semi-logarithmic straight lines. These have the general formula $A = A_0 e^{-k_a t}$. A_0 is the intercept on the zero time axis, A is a point on the line at time t, and k_a is the slope. k is solved for by transposition and substitution in this formula $k_a = 2.3/t \log A_0/A$. The formula for the original curve would then be the sum of its individual components, thus: $P = A e^{-k_a t} + B e^{-k_b t} + C e^{-k_c t} + \ldots$

Each of the semi-logarithmic lines presumably indicates the disappearance or distribution into a particular compartment or pool. Their slopes indicate the rates at which this occurs; the values multiplied by 100 would give this factor in per cent per hour. The intercept of each line with the zero time axis indicates the percentage of the total administered which is to disappear or be distributed into the particular compartment.

The cumulative excretion or "appearance" curve was converted into a urinary disappearance curve by serially subtracting the cumulative excretion from the total excreted. This curve then indicates the amount remaining in the body whose fate it is to be excreted. These curves were then resolved in the manner described.

The expander remaining in the body at any time t is the amount given minus the amount cumulatively excreted to that time. The curve of the

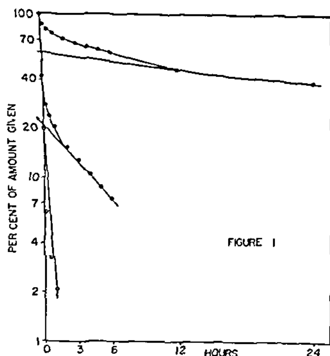


Fig. 1. The plasma disappearance curve (solid circles) resolves into three semi-logarithmic components which are the straight lines. From above down the slopes of these lines are designated k_a, k_f and k_c, and their intercepts A, B and C in Table I.

2 Fat globules were not seen in the peripheral blood samples of an appreciable number of 20 convalescent patients following a challenge by ACTH

3 The results of this study place considerable doubt on the bone marrow theory of origin of embolic fat, and present suggestive evidence that fat emboli arise from soft tissue trauma at the time of surgery or may be a response to stress

REFERENCES

1 Peltier L F Fat embolism Surgery *36* 198 203 1954
2 Davis H L and Goodchild C G Emulsion stability and fat embolism J Chem Educ *13* 478 481 1936
3 Lehman E P and Moore R M Fat embolism Arch Surg *14* 621 662 1927
4 Becke G H Meyer J and Necheles H Fat absorption in young and old age Gastroenterology *14* 80 92 1950
5 Sorce G Experimental research on fat embolism Surg Gyn Obst *72* 207 208 1941
6 *Davis H L and Musselman M M Blood particle agglomeration and fat embolism* Internat Rec Med *167* 439 1954
7 Gauss H Studies in cerebral fat embolism Arch Int M *18* 76 102 1916
8 Peltier L F Fat embolism following intramedullary nailing Surgery *32* 719 722 1952
9 Whiteley H J The relation between tissue injury and the manifestations of pulmonary fat embolism J Path Bact Lond *67* 521 530 1954
10 Peltier L F Personal communication

THE KINETICS OF EXPANDER TRANSFER AND DISTRIBUTION FROM THE PLASMA DISAPPEARANCE CURVES*

WILLIAM METCALF AND LOUIS M ROUSSELOT

The plasma disappearance curves of the expanders studied in this laboratory were found very similar to the curves obtained in studying the kinetics of disappearance of T 1824[1] Both groups of curves were also noted to be quite similar to the published curves of the disappearance of radioiodoalbumin and other tagged substances [3,4] Many of the latter curves compound in nature could be resolved by graphic analysis into 2 or more semi logarithmic components these indicated 2 or more concurrent disappearance rates and distribution compartments Since the expanders are also distributed from the plasma into various compartments or pools graphic analysis of their curves was undertaken to obtain information on the kinetics of their transfer and distribution

METHOD

The data used were those previously obtained on 4 expanders—Laros dextran CSC dextran Knox modified fluid gelatin and Baxter oxypoly gelatin[5,6] Each expander series consisted of 2 groups of subjects 6 to 10

*From the Department of Surgery and the Surgical Research Laboratories of St Vincent s Hospital New York City and the Department of Surgery New York University College of Medicine This investigation was supported by the Medical Research and Development Board Office of the Surgeon General Department of the Army under contract No DA 49 007 MD 199

The authors gratefully acknowledge the technical assistance of Miss Sally Davis

Table 1 The Distribution Factors of Various Expanders

		SLOPES						INTERCEPTS					
		PLASMA			BODY	URINE		PLASMA			BODY	URINE	
		k	k_1	k_2	k_3	k_4	k	C	B	A	D	E	F
Dextran													
(Latos)	60gm	224	180	016	013	1.30	1.61	19.7	22.3	[illegible]	[illegible]	12.1	[illegible]
	30gm	301	[illegible]	034	031	1.31	2.08	16.7	17	[illegible]	[illegible]	10.3	21
	Aver	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	18.2	19.9	61.9	[illegible]	11.2	21.2
Dextran													
(CSC)	60gm	348	60	018	016	[illegible]	1.60	[illegible]	31.8	[illegible]	[illegible]	18.1	[illegible]
	30gm	[illegible]	330	[illegible]	018	1.16	1.43	14.7	30	18	[illegible]	[illegible]	[illegible]
	Aver	[illegible]	31	022	01	1.06	1.7	19.3	32.7	18.1	13.8	17.0	[illegible]
MIC													
	30gm	2.32	313	[illegible]	038	1.33	2.00	31.6	38	30.6	31.0	[illegible]	[illegible]
	1 gm	[illegible]	[illegible]	[illegible]	017	[illegible]	3.18	[illegible]	[illegible]	41.1	36.7	21.1	[illegible]
	Aver	229	331	01	013	[illegible]	[illegible]	30.8	[illegible]	57.1	33.9	21.8	[illegible]
OIC													
	70gm	[illegible]	[illegible]	010	010	40	[illegible]	[illegible]	18	[illegible]	[illegible]	[illegible]	—
	2 gm	[illegible]	[illegible]	[illegible]	[illegible]	3.00	—	40.0	[illegible]	[illegible]	30.0	[illegible]	[illegible]
	Aver	[illegible]	[illegible]	031	036	4.20	3.77	40.0	[illegible]	51.1	32	79	[illegible]

the latter Conversely the slowly disappearing component represents a larger proportion of the dextrans and a smaller proportion of the two gelatins intercept A for the dextrans is 61.9 and 18.1 per cent and for the gelatins is 57.1 and 51.1 per cent.

The body distribution analysis resulted in only 1 semilogarithmic component This component turned out to be practically identical to the slow component of the plasma disappearance curve The slopes of the body components for the 4 expanders were nearly equal to those of the slow components of the plasma curves and the intercepts for both were in agreement within 3 to 4 percentage points (compare k_2 with k_3 and intercepts A with D)

The urine disappearance curve resolved into 2 components Their slopes were somewhat less steep than those of the 2 fast components of the plasma curves However the sums of the intercepts of the 2 components of the urine curve were practically equal to the sums of the 2 faster components of the plasma As was already noted for the plasma components the urine components for the 2 gelatins showed much steeper slopes and much higher intercepts than did the 2 dextrans

DISCUSSION

Generally this study amplified the previous data and confirmed the conclusions previously reached These were that plasma expander retention is directly related to its average molecular weight and urinary excretion is inversely related to this factor Thus the gelatins with molecular weights between 30 and 50 000 were excreted much more rapidly and in greater amounts than the 2 dextrans which have molecular weights of

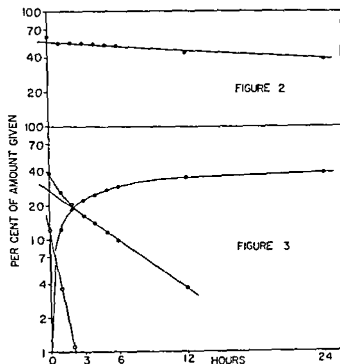


Fig 2 The body distribution calculations result in only one component with slope k_d and intercept D

Fig 3 The cumulative urinary excretion curve (solid circles) is converted by subtraction from the total excreted into a disappearance curve (half open circles) The latter curve resolves into two components with slopes k and k_f and with intercepts F and F

ratio of the amount remaining in the *plasma* to that remaining in the *body* i e (plasma expander remaining) / (100 cumulative excretion) multiplied by the total remaining in the body may be designated as the body distribution curve Its resolution components would indicate the distribution of expander remaining in the body and not destined to be excreted

An illustration of the resolution of the plasma urine and body distribution curves is given in Figures 1 2 and 3 This example is from the group of patients who received 60 gm of Laros dextran The intercepts and calculated slopes for this example will be found in the first row of figures in the table summarizing the results

RESULTS

The data derived from analysis of the various curves in the 4 expander series are given in Table I

The plasma disappearance curves usually resolved into 3 components Occasionally a fourth could be made out but as it depended on only 2 or at most 3 points the third curve was usually not further resolved and it was considered a straight line Comparison of the plasma k s shows the fastest component to be 10 times the moderate one and about 100 times the slowest one

The average slopes for the 2 most rapid components of each expander are similar for the 4 expanders However the least rapid component is slowest for the 2 dextrans (k_a 025 and 022) and fastest for the 2 gelatins (k_a 015 and 034)

The percentile distribution data (intercept section Table I) indicates that a smaller fraction of the dextrans and a much greater fraction of the gelatins is in the rapidly disappearing component intercept C is 18 2 and 19 3 per cent for the former but 30 8 per cent and 16 0 per cent for

SUMMARY

A method of graphic analysis has been applied to the plasma disappearance and urinary excretion curves of 4 expanders. This method resolved these compound curves into their semilogarithmic components and a mathematical formula for each curve could be derived. A more exact formulation of the kinetics of expander disappearance could thus be made and the relation between plasma disappearance and urinary excretion quantitatively defined. Resolution of the plasma curve alone makes it possible to deduce the quantity of expander destined to remain in the body and its rate of disappearance from the circulation. From this curve it is also possible to deduce the amount of expander to be excreted and thus obviate the need for urine collection and analysis.

REFERENCES

1 Unpublished material
2 London I M Cincinnati The Robert Gould Foundation Inc Symposia on Nutrition 2 72 82 1950
3 Berson S A Yalow R S Schreiber S S and Post J Tracer experiments with I¹ labeled human serum albumin Distribution and degradation studies J Clin Invest 32 746 768 1953
4 Walker W C, and Wilde W S Kinetics of radiopotassium in circulation Am J Physiol 170 401 413 1952
5 Metcalf W, and Rousselot L M Some physiologic effects following a dextran infusion in normal subjects In Surgical Forum 1952 Philadelphia W B Saunders Co, 1953 pp 428 433
6 Metcalf W Rousselot L M, Harmon J M and Gilbertson E I The determinants of the efficacy of various expanders in plasma volume expansion and maintenance in normal subjects in Surgical Forum 1953 Philadelphia W B Saunders Co 1954 pp 714 719
7 Solomon A K Symposium on Radioactive Isotopes Equations for tracer experiments. J Clin Invest 28 1297 1307 Nov (pt 1) 1949
8 Cohn W E and Brues A M Metabolism of tissue cultures Method for measuring permeability of tissue cells to solutes J Gen Physiol 28 449 461 1945
9 Metcalf W Rousselot L M and Gilbertson E I The renal clearance of plasma expanders in Surgical Forum 1954 Philadelphia W B Saunders Co 1955 pp 520 523
10 Wasserman K and Mayerson H S Plasma lymph and urine studies after dextran infusions Am J Physiol 171 218 225 1952

55 000 and 65 000, conversely, the dextrans showed greater retention in the plasma and were excreted more slowly and in lesser amounts

Specifically this study by graphic analysis of the logarithmic curves did allow a more exact formulation of the kinetics of expander distribution and disappearance to be made Analysis of the arithmetic curves in the previous studies allowed only a roughly quantitative expression of the kinetics of expander disappearance In this study components of the curves became apparent and they could be characterized by quantitative values In fact a mathematical formula for the plasma or urine curves can be obtained by summation of the expressions for the individual components derived by resolution of the original compound curves

The marked similarity in characteristics of the single component from the body distribution calculation and the slowest component of the plasma curve indicated that they were in fact identical Since elimination of the urinary factor resulted in only one semi logarithmic curve this would imply that the expander remaining in the plasma and not destined to be excreted is distributed into only one body compartment by one mechanism at a given rate Also since the kidneys apparently fractionate the expanders[9] and excrete the molecules with a molecular weight below 70 000 it would appear that this curve characterizes the disappearance in the body of the molecules with a molecular weight above 70 000 Whether this represents phagocytosis or slow metabolism is not known from these experiments

The other two components of the plasma curve represent together that fraction of the expander which will be excreted It may be questioned why their rates of disappearance are different than those of the two urinary components to which they are equivalent and whose sum they equal The rate of loss of these plasma components is the resultant of 3 different rates (1) the loss through the kidneys (2) the loss through the capillary bed and (3) the return into the circulation from the extra vascular area Since there is an equilibrium established between the lymph and the plasma expander[10] the rate of loss from both these compartments would be determined solely by the rate of excretion through the kidneys The rates for the 2 urine components were less than those for the 2 plasma components This is in keeping with Fine s suggestion that this difference might be expected because of the double capillary membrane of Bowman s capsule as opposed to the single layer of the general capillary bed

It is unlikely that the 2 renal components each represent a different process of excretion It is also unlikely that the 2 faster plasma components which represent the plasma fraction to be excreted each represent a different process of exchange across the capillary membranes It is more likely that the components represent the limits of resolution of this method of analysis in the very rapidly changing portion of the curves With many more points available and a graph drawn to very large scale the curves could no doubt be resolved into many more components

Since the intercept of the slowest component of the plasma curve represents the amount of expander to remain in the body the difference between that value and the total would represent the amount to be excreted Resolution of the plasma disappearance curves therefore allows the calculation of the amount of expander that will be excreted and collection and analysis of urine for that determination becomes unnecessary

Pup 6285 Received 12.5 gm. of oral horse meat and liver in addition to the basal non-protein diet. Total daily calories offered was 1,000.

Pup 6277 Received 12.5 gm. of plasma proteins as intravenous plasma daily and only the non-protein diet orally. Total calories offered was 1,000.

Pup 6376 Received only the non-protein diet orally. No plasma was given. Total calories offered was 1,000.

Nitrogen balance studies were conducted continuously with a 28 day control period beginning at the eighth week of life and ending 88 days later when the experiment was terminated. From day 28 to day 88 was the test period during which each pup was on its test feeding schedule. Complete blood counts, plasma protein determinations and urine analyses were made twice a week on each pup. Daily weights were recorded.

For the first 5 weeks of the test period all 4 pups ate well. Thereafter the pup (6376) given neither oral nor intravenous protein consumed only 7… per cent of his diet.

RESULTS

In Table 1 are shown the results of the nitrogen balance studies on each pup during the 60 day test period. It will be noted that the large positive balance of the control period was sharply reduced when the protein consumption was curtailed from 95 gm. during the control period to 12.5 gm. per day allowed during the test period. However, the nitrogen balance continued to be on the positive side each day of the test period.

Two points are of interest from the blood studies. First, anemia developed within a week after the repeated daily administrations of plasma was begun. When this became severe, transfusion of packed red cells was given and then the plasma transfusion schedule resumed. Second, a pronounced hyperproteinemia resulted when 250 to 275 ml. of plasma were given daily; this almost disappeared, however, when the daily volumes of transfused plasma were reduced to 200 ml.

Figure 1 is a photographic comparison of the appearance of pups 6277 and 6376 on the final day of the study. Neither of these pups received oral protein but 6277 received 12.5 gm. of plasma daily for the 60 days of the experiment. His general appearance was the same as that of his litter mate (6285) given 12.5 gm. of oral protein for the same period of time. Of

Table 1. Weight Gain and Nitrogen Excretion of Dogs Given 12.5 gm. of Protein Daily and the Controls (Litter B)

DOG NO.	ROUTE	TYPE PROTEIN	DAILY AMT.	WEIGHT		NITROGEN BALANCE IN GRAMS DAILY AVERAGE		
				BEG.	END	INTAKE	EXCRETION	STATUS
6275	Oral	Horse Meat	95 gm.	6.0	8.8	15.20	8.20	Pos. 7.00
6285	Oral	Horse Meat	12.5 gm.	6.5	6.8	1.60	0.50	Pos. 1.10
6277	Intravenous	Plasma	12.5 gm.	6.7	7.7	1.60	0.47	Pos. 1.13
6376	—	None	0.0	6.0	4.9	0.03	1.03	Neg. 1.00

COMPARATIVE GROWTH STUDIES OF PUPPIES RECEIVING THE SAME QUANTITIES OF PROTEIN AS ORAL LIVER AND HORSE MEAT WITH THOSE RECEIVING INTRAVENOUS PLASMA*

EDWARD A STEMMER LOUIS R HEAD AND J GARROTT ALLEN

Seven years ago[1] data were presented from this laboratory which demonstrated that hypoproteinemia in depleted patients could be corrected by the daily administration of a liter or more of plasma intravenously While others confirmed these observations a number objected to our proposal that the transfused plasma is metabolized at a rate useful to the nutrition of such patients The evidence we presented in 1948 favoring the nutritive usefulness of plasma in man was substantial although admittedly indirect. The plasma protein concentrations in these patients returned to normal values The nitrogen balance became positive to a remarkable degree (5 to 15 gm per day) and there was no evidence of a delayed or latent nitrogen loss The patient s general condition improved

The objections to our conclusions centered chiefly about 2 points

1 That a positive nitrogen balance while achieved during the transfusion period did not necessarily imply plasma utilization

2 That if plasma were metabolized, it would not likely be utilized at a rate sufficient to satisfy the body s needs for nutrition

Because of the general concern and eventual abandonment of plasma transfusion due to the high incidence of homologous serum jaundice from dried plasma we could not proceed further with this problem until a plasma free of such risk could be prepared and tested With a safe plasma now available- we have returned to our original problem

To avoid objections of technical nature to our current study we decided upon the simple observation of comparing photographically the rates of growth of litter mate puppies given different protein sources but otherwise identical diets

EXPERIMENTAL PLAN

Four litter mate mongrel puppies housed in our laboratory since birth were fed the same quantity of our laboratory diet from the second to third month of life Thereafter they all received the same basic non protein diet adequate otherwise in fat carbohydrate minerals and vitamins To this basic non protein diet was added horse meat and liver for 2 of the pups No oral protein was added to the diet of the remaining 2 pups The 4 pups were each treated differently with respect to the protein allowed and its route of administration The schedules for each pup during a 60 day test period was

Pup 6275 Received 95 gm of oral horse meat and liver in addition to the basal non protein diet Total daily calories offered was 1 000

*From the Department of Surgery University of Chicago School of Medicine Chicago Illinois. This work was conducted under Contract No DA-49-007 MD 93 with the Surgeon General s Office of the Department of the Army

we have previously reported for man.[1] Its cause is not known but is more likely from slight but continuing hemolysis than from plasma dilution. There is also suggestive evidence that the production of red cells may be impaired by the repeated transfusion of plasma obtained from normal non-anemic dogs.

CONCLUSIONS

Transfused plasma is as useful to growth and nutrition in litter mate puppies as the same quantity of horse meat and liver fed by mouth.

REFERENCES

1. Allen, J. G., Bogardus, G., Egner, W., and Elghammer, D. B.: The correction of hypoproteinemia by the administration of plasma and blood. Surg. Gyn. Obst., 86:601, 1948.
2. Allen, J. G.: The safety of liquid plasma. Surg. Gyn. Obst., 100: 19 ... 19

THE EXPERIMENTAL RELATIONSHIP OF MALNUTRITION TO PROTEIN RESERVE AND TOLERANCE TO A MAJOR OPERATIVE LOAD*

DANIEL A. ALLENDE

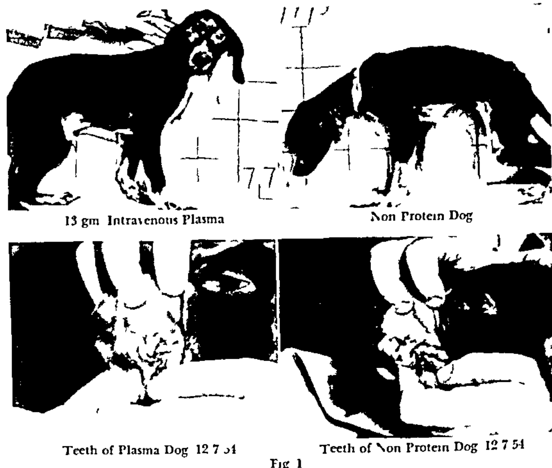
The plasma proteins are an important constituent of the total protein mass of the animal body and as such are in metabolic equilibrium with the tissue proteins. They have a close relationship with the protein and caloric content of the diet and hypoproteinemia has been recognized as a cause of several syndromes and complications following surgery.[1, 2] The difficulties inherent in evaluating both the blood protein levels and body protein reserves in patients before and after major surgical procedures has long been appreciated. This study was undertaken to determine whether the mortality rate and the fall in blood protein levels after an operation are significantly increased in dogs whose blood protein levels were normal at operation but in which the diet preoperatively had been deficient in protein and caloric intake. It was intended to simulate conditions in which patients who have had inadequate dietary intake for a few weeks but with still relatively normal blood protein levels, are submitted to a major surgical procedure.

METHODS

A "basic diet" was planned for this study which contained 80 calories per kg., 25 per cent of which was protein. In order to deplete the experimental group of dogs, a "depletion diet" was devised which consisted of only one-tenth of the basic diet or 8 calories per kg. This depletion diet, with insufficient quantities of every constituent of a normal diet, resembles the dietetic regimen of some patients who reduce their food intake considerably during a period of a few weeks without causing significant changes in their blood protein levels. In order to determine how long dogs could be fed

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* From the Department of Surgery, University of Illinois College of Medicine. Aided by a grant from the Graduate School, University of Illinois. Credit is hereby extended Mr. Everett Hoffe for technical assistance.



13 gm Intravenous Plasma — Non Protein Dog

Teeth of Plasma Dog 12 7 54 — Teeth of Non Protein Dog 12 7 54

Fig 1

interest also is the poor development of the teeth in the dog given neither oral nor intravenous protein (6376) its deciduous teeth are still present whereas the permanent teeth are present in the plasma infused litter mate as well as in the other 2 litter mates given oral protein

Once the test period was completed the pups were again placed on a standard diet containing 95 gm of protein daily Each grew well and little or no difference is discernible at the end of 1 year

DISCUSSION

The gain in body weight was greater by 1 kg for the dog receiving daily intravenous plasma compared to that receiving the same quantity of protein as horsemeat and liver by mouth However 12 5 gm of protein whether by mouth or as plasma by vein did not permit maximum growth as indicated by the larger weight gain exhibited by the pup receiving 95 gm of protein by mouth daily during the test period

The value of intravenously administered plasma as a source of protein adequate for growth and nutrition seems clearly established by these studies alone However this experiment has now been repeated using a test period of 90 days and the same favorable response observed in a second litter of puppies That these data apply to man seems very likely for they compare favorably in all points where previous studies on intravenously administered plasma in man were similar only the human growth factor could not be studied or tested in man and hence cannot be compared

The anemia associated with the repeated plasma transfusions in dogs

MEAN PERCENT PROTEIN VALUES, WEIGHT AND BLOOD VOLUME OF FOUR DOGS THAT DIED AND FIFTEEN DOGS THAT SURVIVED A STRESS OPERATION AFTER FOUR TO FIVE WEEKS NUTRITIONAL DEPLETION

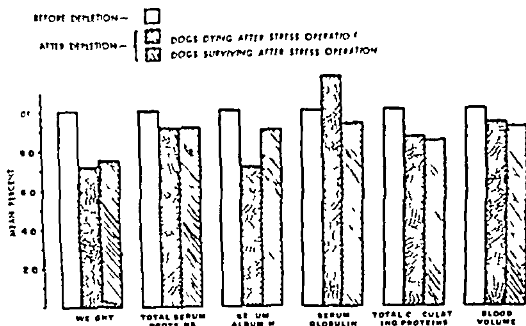


Fig 1 Subgroups of depleted dogs that died (stippled columns) or survived (shaded columns) are compared with their pre-depletion levels of (left to right) weight total serum proteins serum albumin serum globulin total circulating protein and blood volume

RESULTS

The experimental group consisted initially of 21 dogs 2 of which were discarded during the experiment leaving 19 animals to be finally evaluated Four of the 14 control dogs were eliminated leaving 10 to be compared with the experimental group Of the 6 animals eliminated 4 were discarded because illnesses developed and 2 because of minor surgical accidents These factors no longer allowed accurate comparison of the affected dogs with the others As shown in Table 1 there were no deaths among the control dogs after the stress operation In the experimental group 1 dog died after being anesthetized just as surgery was to begin The other 3 deaths occurred at 24 30 and 36 hours after operation Liver biopsy at operation was performed on 5 experimental and 4 control dogs In the depleted as well as the control animals the histological appearance of the liver was normal

The experimental group may be divided into subgroups of dogs those that survived and those that died after the stress operation Data on these animals is shown in Fig 1 comparing the mean of weights and blood protein values for each subgroup after depletion with their normal values before depletion The 10 control dogs are compared with 15 post stress survivors over a 13 day period after the stress operation Total serum protein and albumin values are compared in Fig 2 while total circulating protein levels are contrasted between these two groups in Fig 3

DISCUSSION

Obviously weight loss should be a good index of the degree of depletion in dogs maintained on a low caloric intake for several weeks A progressive

the depletion diet before a serious alteration in their serum proteins was produced 2 scout groups of dogs were studied Results on the 11 dogs so studied indicated that dogs could be fed the depletion diet for 4 or 5 weeks

In the main experiment adult mongrel dogs of both sexes were used They were all initially in good health were immunized against rabies dipped and dewormed The animals ranged in weight from 8 to 20 kg All were observed for 10 to 15 days before any tests were begun and were fed the basic diet during this time to stabilize their protein reserves Twenty one dogs comprised the experimental group and 11 dogs were used as controls In the experimental group after stabilization on the basic diet 6 dogs were fed the depletion diet for 34 days and the other 15 received it for 28 days At the end of these periods they were submitted to a standard surgical operation Control dogs were operated upon after the stabilization period The operation was intended to be a major one which would impose severe surgical stress but which could be tolerated by normal dogs with a minimal or no mortality rate

Accordingly a standard reproducible procedure was devised consisting of cholecystectomy splenectomy and the removal of 18 cc blood/kg body weight This blood loss was known to be heavy but below shock level This stress operation was done aseptically with anesthetic agents carefully measured and administered Three to 4 hours before surgery morphine sulfate 30 mg was given subcutaneously and sodium pentobarbital 22 mg / kg was given 30 minutes before operation An endotracheal tube was used during each operation to insure a free airway The surgical procedure required approximately 75 minutes in each case Postoperative feeding consisted of milk on the first day one half of the basic diet on the second day and the full basic diet on the third day and subsequently All surviving dogs were sacrificed 21 days after the stress operation except for the last 4 in the experimental group which were sacrificed 13 days postoperatively

Weight total serum proteins serum albumen serum globulin plasma volume and total circulating protein were determined (1) before depletion (2) weekly during the period of depletion (3) on days 3 and 1 before the stress operation and (4) on days 1 3 6 and 13 after the stress operation Weight was measured in kilograms and the other determinations expressed in terms of body surface area to make more accurate comparisons among dogs of different weights and sizes Protein analyses were performed using a modified micro Kjeldahl technique and Howe s salting out method Plasma volume was determined with Evans blue (T 1824) dye Thiocyanate space measurement of extra cellular fluid volume was done on some of the animals but is not included in this report

Table 1 Mortality Figures for the Experimental and Control Groups

GROUP	NUMBER OF DOGS	DEATHS AFTER ANESTHESIA	DEATHS AFTER OPERATION	TOTAL DEATHS
Experimental Group	19	1	3	4
Control Group	10	0	0	0

the most part below normal. The serum albumin levels varied widely above and below normal. In more than half of the animals the serum albumin increase was at the end of the depletion period. This wide variation can perhaps best be explained by an increase in the alpha globulin fraction which is difficult to separate from the albumin fraction by the laboratory methods employed. The increase in alpha globulin fraction during deple-tion has been reported by other workers[3].

With regard to the mortality figures in Table I there were 4 deaths in the depleted group but no deaths among the controls. This mortality rate of 21 per cent in the experimental group did not prove to be statistically significant but a definite trend seems indicated. Shock was the apparent cause of death in the depleted dogs. At autopsy the only important gross finding was marked atrophy of the liver and myocardium. In contrasting the sub-groups of surviving and deceased depleted animals following the stress operation it is seen in Fig. 1 that most of the values after depletion are similar in the two sub-groups. However there is no reversal of the albumin globulin ratio after depletion in the surviving group but a marked reversal of this ratio in those dogs that died after operation. The serum albumin among the postoperative deaths had declined by 29 per cent from its level before depletion and the serum globulin had increased 118 per cent. The mean body weight in this sub-group had also been reduced by 29 per cent. This albumin globulin reversal has a definite clinical implication and may be related to hepatic insufficiency.

The postoperative data for the depleted survivors in Figs. 2 and 3 shows a more marked fall in the total serum protein serum albumin and total circulating protein on the first postoperative day than occurred in the control group. Blood volume and plasma volume levels for the depleted survivors also fell more sharply. By the thirteenth postoperative day however total serum protein total circulating protein and plasma volume had almost returned to pre-depletion normal in the experimental group. There was a definite gain in weight in this group although serum albumin levels remained practically unchanged.

The control dogs followed a course that resembled the depleted survivors. The values in all categories dropped on the first postoperative day, the total circulating protein more so than the others. By the third day the plasma volume was above normal and the total circulating protein level had improved considerably. By the thirteenth day the total serum protein and serum albumin had not returned to preoperative levels and there was a slightly reversed albumin globulin ratio. Total circulating protein and plasma volume had returned to normal level. The control dogs thus showed a more delayed type of response their protein levels returning to normal more slowly than those of the experimental dogs. This difference might be explained either by a more efficient alarm response or by a more efficient assimilation of food in the latter. These two factors may well be related. Previous workers[4] have shown that following operation depleted patients reveal a smaller negative nitrogen balance than do patients with normal nutrition.

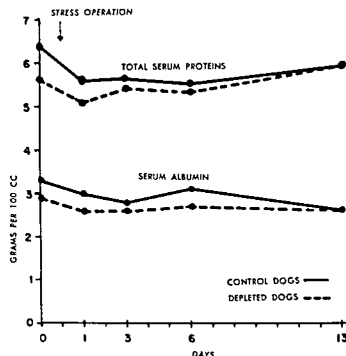


Fig 2 Total serum protein and serum albumin levels after operation are shown for the depleted survivors (broken lines) as compared with the control dogs

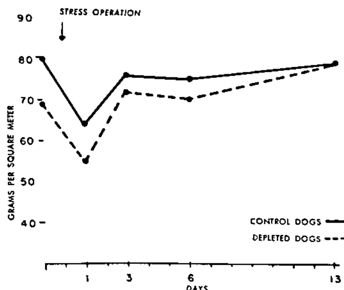


Fig 3 Total circulating protein levels after operation are shown for the depleted survivors (broken line) as compared with the control dogs

loss of weight was observed during the period of depletion in the 15 dogs surviving the stress operation weight loss was uniform averaging 25 per cent of initial body weight at the end of 4 to 5 weeks as shown in Fig 1 The same graph shows the mean values for total serum protein and total circulating protein both of which were slightly reduced after depletion There was moderate variation in these values in individual dogs but they were for

Surgical Infection

ENHANCED PRODUCTION OF ANTIBODIES BY FOCAL RADIATION*

JOHN B GRAHAM RUTH M GRAHAM AND SIDNEY LESKOWITZ

Exposure of the entire body to ionizing radiation will suppress antibody production Forty years ago Hektoen[1] recognized this principle and it has been repeatedly confirmed since then[2] Such irradiation will not appreciably alter the immune response that was begun 4 days or more before but more recently initiated immunity is deleteriously affected

In a search for an explanation of the beneficial effect of radiotherapy in cancer it was found that local radiotherapy not only failed to inhibit antibody production but actually seemed to stimulate it[3]

Young adult rabbits weighing about 5 pounds were given an intracutaneous injection of sheep red blood cells in the lateral aspect of the right thigh In some that thigh was exposed to 1000 r (2000 kv) one half hour after the injection Hemolysins were determined at weekly intervals A maximum level was observed at the second week

In the first experiment 0 1 cc of whole sheep blood diluted 1 8 was used Fourteen animals received the antigen alone and 10 received the antigen followed one half hour later by 1000 r As one can see in Figure 1 the irradiated animals had a higher level of hemolysin than the unirradiated animals Of the 14 animals receiving antigen but no radiation 6 had a titer

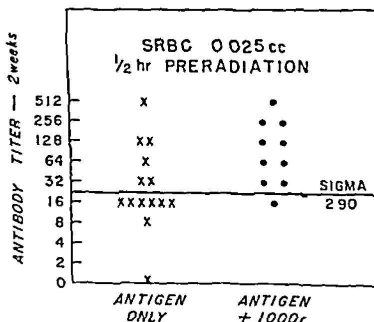


Fig 1

From the Vincent Memorial Hospital the Gynecological Service of the Massachusetts General Hospital Boston Mass and Massachusetts Institute of Technology Cambridge Mass Supported in part by funds from the American Cancer Society

SUMMARY

Nineteen dogs were depleted on a low caloric diet for 1 to 5 weeks Their blood protein levels dropped 9 per cent below their initial value and their weight diminished by 25 per cent A stress operation consisting of chole cystectomy splenectomy and bleeding of 18 cc blood per kg was then performed Various blood protein determinations were made before and during depletion and on several days after surgery to the thirteenth day Three dogs died after the stress operation and a fourth after being anesthe tized The only real difference between the depleted dogs that survived or died after operation was a marked reversal of the albumin globulin ratio in the latter group Ten control dog received the same operation without mortality Accordingly albumin globulin reversal might be considered a reflection of poor operability In the postoperative period the control dogs actually exhibited a slower return of their protein levels to normal than did the depleted survivors This implies that depleted animals may develop a more efficient response to a major surgical procedure although they may be less likely to survive the immediate effect of the operation

REFERENCES

1 Allison J B Some relationships between diet protein stores and plasma proteins Symposia of Nutrition Vol II Ed by J B Youmans Springfield Ill Chas C Thomas 1950 pp 123 137

2 Ariel I M The internal balance of plasma protein in surgical patients Surg Gyn Obst 92 405-414 1951

3 Elman R Clinical problems in hypoproteinemia due to protein deprivation Symposia of Nutrition Vol II Ed by J B Youmans Springfield Ill Chas C Thomas 1950 pp 138 154

4 Moore F D and Ball M R The metabolic response to surgery American Lectures in Surgery Ed by M F Debakey and R G Spurling Springfield Ill Chas C Thomas 1952

Surgical Infection

ENHANCED PRODUCTION OF ANTIBODIES BY LOCAL RADIATION*

JOHN B. GRAHAM, RUTH M. GRAHAM AND SIDNEY LASKOWITZ

Exposure of the entire body to ionizing radiation will suppress antibody production. Forty years ago Hektoen[1] recognized this principle and it has been repeatedly confirmed since then[2]. Such irradiation will not appreciably alter the immune response that was begun 4 days or more before, but more recently initiated immunity is deleteriously affected.

In a search for an explanation of the beneficial effect of radiotherapy in cancer, it was found that local radiotherapy not only failed to inhibit antibody production but actually seemed to stimulate it[3].

Young adult rabbits weighing about 5 pounds were given an intracutaneous injection of sheep red blood cells in the lateral aspect of the right thigh. In some that thigh was exposed to 1000 r (2000 kv) one half hour after the injection. Hemolysins were determined at weekly intervals. A maximum level was observed at the second week.

In the first experiment 0.1 cc. of whole sheep blood diluted 1:8 was used. Fourteen animals received the antigen alone and 10 received the antigen followed one half hour later by 1000 r. As one can see in Figure 1, the irradiated animals had a higher level of hemolysin than the unirradiated animals. Of the 14 animals receiving antigen but no radiation, 6 had a titer

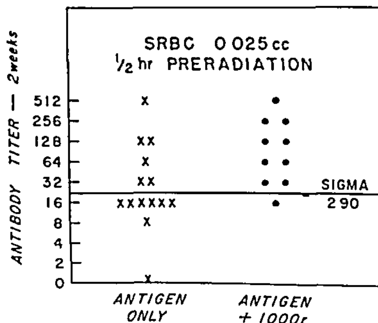


Fig. 1

* From the Vincent Memorial Hospital, the Gynecological Service of the Massachusetts General Hospital, Boston, Mass., and Massachusetts Institute of Technology, Cambridge, Mass. Supported in part by funds from the American Cancer Society.

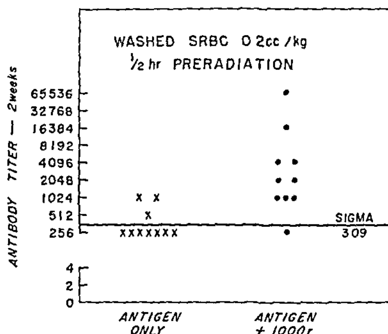


Fig 2

of more than 1 16 while 9 of the 10 irradiated animals had a titer above that level Statistical evaluation of this distribution shows a sigma of 2 90

In the second experiment, a similar procedure was followed but a much larger dose of antigen was used i e 0 2 cc of washed sheep red blood cells per kg of body weight As one might expect the resulting titers of antibody were appreciably higher There were 10 which received antigen only and 3 of these showed a level of more than 1 256 while 9 of the 10 which received antigen plus radiation had a titer above this level The difference in distribution here has a sigma of 3 09 Four of the animals listed as receiving antigen only actually received the antigen in the right thigh and were irradiated on the left thigh These animals had slightly lower levels of antibody than their fellow controls which received no antigen at all

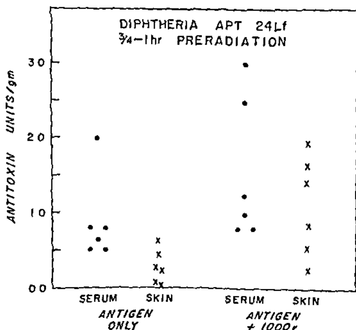


Fig 3

A third series was run using diphtheria toxoid and ¾ to 1 hour later a small volume of tissue (60 cc.) about the injected site was irradiated with 1000 r. Twenty days later the animals were killed. The level of antitoxin in the serum and in the skin surrounding the injection site was determined. As can be seen in Figure 2, only 1 animal which received antigen alone had a serum level of more than 0.8 unit/cc., while 4 of the 6 receiving both antigen and radiation exceeded this level. Significantly, a similar difference is noted in the skin, for none of the controls had a level exceeding 0.8 unit/gm., while 4 of the 6 irradiated animals had a higher titer than that.

These data are regarded as evidence that local radiation not only fails to inhibit antibody formation but actually may stimulate it. It is possible that this mechanism is of importance in the treatment of cancer by ionizing rays.

REFERENCES

1. Hektoen, L. The influence of x-rays on the production of antibodies. J. Infect. Dis., 17: 415-422, 1915.
2. Dixon, F. J., Talmage, D. W., and Maurer, P. H. Radiosensitive and radioresistant phases in the antibody response. J. Immunol., 68: 693-700, 1952.
3. Graham, J. B., Graham, R. M., Neri, L., and Wright, K. A. Enhanced production of antibodies by local irradiation. I & II. J. Immunol. (in press).

THE SERUM PROPERDIN TITERS IN SURGICAL PATIENTS*

JERRET W. BENSON, WILLIAM L. ABBOTT, WILLIAM D. HOLDEN AND STANLEY LEVEY

The nature of specific natural immune mechanisms in man and animals still remains obscure after many years of intensive study. Pillemer and associates[1,2] recently isolated a protein from normal mammalian serum which appears to be a factor in certain natural immune processes. This protein, named *properdin*, is a euglobulin comprising not more than .03 per cent of total human serum proteins. Properdin, complement and magnesium have been identified and characterized as the constituents of the properdin system, a natural defense mechanism of blood. The activities of this system do not require specific antibodies and are unrelated to known antigen-antibody reactions. This system kills certain bacteria, neutralizes some viruses and lyses certain abnormal erythrocytes.[1,2,3,4] Properdin appears to function in the absence of specific antibody as a required accessory to the destructive action of normal serum complement against a variety of bacteria.[5]

Marked alterations in the serum properdin levels of laboratory animals have been induced by (1) total body irradiation,[5] (2) the intravenous and intraperitoneal administration of certain carbohydrate complexes having the specific property of combining with properdin,[6,7,8] and (3) experimental shock.[9] By these methods an inverse correlation has been demonstrated

*From the Department of Surgery, Western Reserve University School of Medicine and the University Hospitals of Cleveland, Cleveland, Ohio. This work was supported in part by a grant from the National Institutes of Health, U.S. Public Health Service (A 760 (C3)).

between serum properdin titers and susceptibility to infection with certain bacteria.[7-10]

Human serum properdin titers normally range from 4 to 8 units per ml.[1] and tend to remain quite stable in a given individual in the absence of disease. The purpose of this study was to detect alterations of serum properdin concentration in surgical patients which might be induced by therapy or disease and to evaluate the significance of such changes.

PROCEDURE AND METHODS

Material for this report is derived from the study of patients from the Surgical Divisions of the University Hospitals of Cleveland who have sustained a variety of surgical disease processes and operative procedures. Serial determinations of the serum properdin concentrations of these patients have been performed by a method published elsewhere.[1] Serum samples were separated from fresh venous blood after clot formation by centrifuging and stored at −15 C. Properdin assays were then performed when the series of samples was complete for each patient. Deviations in the serum titer of 50 per cent or more in a given subject are regarded as significant.

A systematic investigation of factors related to trauma, surgical treatment and infection which might influence the properdin system is being carried out. This is a preliminary report of findings in the first 30 patients who have been studied.

RESULTS

Preliminary observations were made on patients undergoing elective operative procedures under general or spinal anesthesia. The combined effects of various types of anesthesia and subsequent operative trauma of varying magnitude have not produced significant alterations in the serum properdin titer detectable in 10 patients studied according to varied sampling routines designed to detect transient changes. In the absence of apparent complications, no marked changes have been noted in the serum titers of these patients during the first postoperative week. The specific operative procedures performed on these patients include cholecystectomy, gastrectomy, herniorrhaphy, adrenalectomy and splenectomy.

Certain hormonal factors which influence the host's resistance to trauma and infection are known to be mediated through the adrenal cortex. Selected patients were studied in whom evidence of such influences acting on the properdin system might be expected.

Limited observations were made on 3 patients who sustained trauma resulting in shock. Two who died of severe trauma and hemorrhage within 3 hours had normal serum properdin concentrations 1 hour after injury. A third patient who developed shock 10 hours following traumatic rupture of the spleen with intraperitoneal blood loss exceeding 2 liters, had a serum properdin titer of 8 units per ml. Following removal of the spleen, he developed severe atelectasis resulting in pneumonitis, accompanied by a fall in properdin titer to 2 units per ml, which persisted without significant deviation for 14 days.

No significant changes in serum properdin titer were noted in 2 patients before and after total adrenalectomy while being maintained on hydrocor

tisone. One previously adrenalectomized patient failed to develop significant alterations in properdin titer during or after hydrocortisone withdrawal for 96 hours. A five-fold increase of the maintenance steroid therapy for 4 days failed to elicit changes in the properdin titer of a similar patient.

Suggestive evidence resulting from the study of animals subjected to total body irradiation indicates that one of the anatomical sources of properdin may be the spleen. Four patients have been studied before and after elective splenectomy performed for thrombocytopenic purpura. Only 1 of these patients exhibited a significant depression of the serum properdin titer, first detected 21 hours postoperatively and persisting for 1 week. A fifth such patient who was subjected to emergency splenectomy was described earlier. A febrile episode occurred in both patients who exhibited depressions of the properdin titer. Interpretation of findings in these patients must await further study.

Abnormal Properdin Titers. Abnormal serum properdin titers have been encountered in a number of surgical patients who had various complications. Such abnormalities have consisted of both high and low titers which have been quite variable in duration.

Marked depressions of the serum properdin titer have been noted in 3 patients who developed atelectasis following abdominal surgery and in whom radiological evidence of increased density of lung fields was demonstrated. Other patients have exhibited depressions in titer in association with pulmonary changes plus other factors which prohibit critical evaluation. Two patients in whom changes in properdin concentration coincided closely with pulmonary changes are presented in Figure 1.

One patient (F. A.) developed atelectasis following subtotal gastrectomy which reached maximal severity on the second postoperative day. Bacteriologic studies showed pneumococci in the sputum. This patient received no

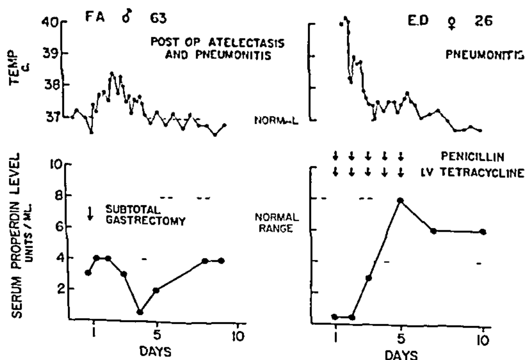


Fig 1

antibiotic and with the subsidence of fever made an uneventful recovery

Another patient E D developed severe bronchopneumonia in one of two remaining lung lobes 6 weeks after a second pulmonary resection for bronchiectasis Dyspnea, cyanosis and temperatures of 10° C necessitated re admission to the hospital Bacteriologic studies revealed only Staphlo coccus aureus coagulase positive in the sputum Serum properdin concen trations when charted with the temperature demonstrate an inverse relationship during the acute process Temperature elevations have been noted in other patients without changes in properdin concentration

In addition to pulmonary complications after surgery depressions of the serum properdin titer have been noted in association with paralytic ileus peritonitis gastrointestinal bleeding and surface wound infections Such deviations of serum titer below the normal range have not persisted in most patients beyond the period of clinical manifestations of the disease Since the significance of such changes is unknown and the number of patients studied is insufficient to reflect the true incidence of such alterations in the serum titer it seemed appropriate to examine a few patients who had demonstrated sustained abnormalities of serum properdin concentration persisting for several weeks

Sustained serum titers above 200 per cent of normal have been encoun tered in 3 patients 2 of whom are represented in Figure 2 Patient S S was admitted with a bullet wound of the left shoulder which resulted in forma tion of an arteriovenous fistula The serum properdin concentration was first determined 48 hours following injury Operative closure of the fistula was accomplished after 20 days and this patient s entire hospital course was uncomplicated The second patient C S was noted to have generalized

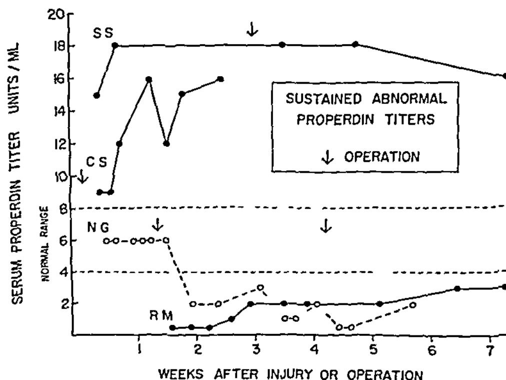


Fig 2

peritonitis when a perforated duodenal ulcer was plicated. Serum properdin titers increased to 16 units per ml. within 6 days. A third patient maintained consistently high serum properdin titers ranging from 18 to 21 units per ml. for 2 months after perforation of a sigmoid diverticulum and partial colectomy. A fecal fistula had formed after 1 week which closed spontaneously after 11 days.

Subnormal serum properdin titers persisted in 2 patients (Fig. 2) for more than 6 weeks. One patient, R. M., sustained second and third degree burns of over 40 per cent of the body surface, followed by persistent infection of surface wounds and a prolonged febrile response. A second patient, N. C., developed partial intestinal obstruction. After abdominal exploration and lysis of adhesions, hydrocortisone was instilled intraperitoneally. His subsequent course was marked by persistent gastrointestinal hemorrhage accompanied by atelectasis, paralytic ileus, and severe esophagitis associated with the use of a Levine tube. A gastroenterostomy with vagotomy was performed which was followed by further depression of a low serum properdin titer. The multiple factors co-existing in this patient are too complex for explanation of the low serum properdin titers. However, the fact that this patient, who exhibited several complications which are common to surgical patients, did not exhibit a normal serum properdin concentration until 4 months after the first operation may be of significance. The failure of multiple transfusions of whole blood (7500 ml. within 12 days) to restore a normal properdin concentration in this patient is consistent with similar observations made in the dog during hemorrhagic shock.[9]

DISCUSSION AND SUMMARY

The preliminary findings herein reported are of insufficient scope to permit conclusions regarding the relationship of changes in serum properdin titer and the general response of the patient to diseases or operations. Suggestive evidence is provided by the study to indicate that sustained deviations of the serum properdin titer do not commonly occur as an immediate effect of anesthesia, operative or accidental trauma, removal of the adrenal glands, or removal of the spleen.

The relationship of changes in serum properdin titer and infection appears to be worthy of further investigation. Impressive changes in the properdin titers of patients with inflammatory processes of the lungs or peritoneum have been encountered regularly in this group, although fever has been noted in other patients without changes in the properdin titer. Factors which frequently co-exist, the separate effects of which may be mutually obscured in many postoperative patients, are atelectasis and paralytic ileus.

Sustained abnormal serum properdin titers in both high and low ranges have been detected in certain patients for periods as long as 8 weeks or more. The significance of these findings as well as the exact relationship of properdin titers to the associated disease processes has not been determined.

These preliminary observations made in a small group of patients are compatible with findings previously reported from the study of laboratory animals which ascribe a major role to the properdin system in resistance to infection. Marked alterations in the properdin concentration of human serum have been demonstrated in association with certain inflammatory

antibiotic and with the subsidence of fever made an uneventful recovery

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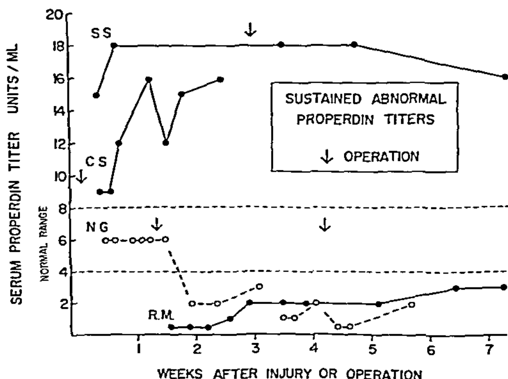


Fig 2

by the enzymes but by their end products. Menkin has described Leukotaxine and Duthie and Chain certain polypeptides, the former is said to attract leucocytes while the latter to increase capillary permeability.

PREVIOUS STUDIES

Recognition of a trypsin-like substance in exudates by Achalme dates from 1899. In 1911 he also described antitrypsin in the serum. Jochmann and Muller found the amount of this protease differed in leucocytes of various species. Opie showed it was very active in human leucocytes. Wright demonstrated purulency decreased in wounds when the antitryptic titer of serum increased and in it only streptococci and staphylococci would grow.

Enzymes associated with the deposition and lysis of fibrin were observed by Barker as early as 1908. The leucoprotease mentioned will of course dissolve fibrin but only when released from leucocytes. Christensen extracted with chloroform still another fibrin dissolving enzyme—plasmin from human serum. Tillett *et al* showed that plasmin was converted to its active form by streptokinase. Barnes found rabbit leucocytes contained cathepsin, nuclease, amylase, lysozyme and adenosinase but not trypsin or erepsin (peptidase). Their lymphocytes contained the same though less active enzymes. After burning, Zamecnik *et al* recovered peptidases in the lymph of dogs[11].

Lysozyme shown by Fleming to lyse certain bacteria was demonstrated in granulations by Prudden *et al*[12] and later Hiatt *et al*[13] showed again that it was in leucocytes.

Most enzymes investigated have been proteolytic and catabolic. Anabolic enzymes have not been reported principally because of lack of methods.

METHOD

Originally the presence of enzymes in granulations was to be determined but reinvestigation of those found in the exudative phase was necessary to demonstrate change. Every attempt was made to exclude enzymes elaborated by bacteria for many bacterial toxins have been shown to be enzymes. Thus either turpentine abscesses or croton oil ur sics[?] were made and granulations were treated with antibiotics. Pus was withdrawn on the fifth day after the abscess formed and it was suspended in water and tested for enzymes together with the serum of the same animal.

To study the granulations a square wound (2.5 cm.) was made aseptically on the ears of rabbits. It was dressed with an ointment (Amerchol H9) containing 1000 units of penicillin. The wound was redressed on the fourth, fifth and sixth days. On the seventh day the granulations were cleaned with saline and scraped from the wound by means of a sharp knife and suspended in a small amount of water. Minimal bleeding was encountered. A concentrated solution of granulations was made in order to determine qualitatively the character of the enzymes present. Serum was obtained at the time.

To show the presence of anabolic enzymes, granulations were used to dress a fresh wound made on the opposite ear of the same rabbit. Sections were made on the first, second and third days of healing to see if capillaries sprouted earlier than on the controls.

Methods of Enzyme Determinations. For the amylase 1 per cent starch was incubated with the granulations or pus for 12 hours. Iodine was added

processes and large blood losses The significant implications of these findings remain to be identified by more extensive observations

The authors wish to thank Drs Louis Pillemer and C F Hinz Jr Leona Wurz and Jean Hower for aid and assistance in this work

REFERENCES

1 Pillemer L Blum L Lepow I H Ross O A Todd E W and Wardlaw A C The properdin system and immunity I Demonstration and isolation of a new serum protein properdin and its role in immune phenomena Science *120* 279 285 1954
2 Pillemer L The properdin system Tr N York Acad Sc *17* 526 530 1955
3 Wardlaw C C Blum L and Pillemer L Bactericidal activity of the properdin system in human serum Fed Proc Balt *14* 480 481 1955
4 Hinz C F Jr Jordan W S Jr and Pillemer L Properdin A specific hemolytic factor in paroxysmal nocturnal hemoglobinuria J Laborat Clin M *44* 811 812 1954
5 Ross O A Moritz A R Walker C J Wurz L Todd E W and Pillemer L The role of the properdin system in whole body irradiation Fed Proc Balt *14* 496 1955
6 Pillemer L and Ecker E E Anticomplementary factor in fresh yeast J Biol Chem *137* 139 142 1941
7 Pillemer L and Ross O A Alterations in serum properdin levels following injection of zymosan Science *121* 732 733 1955
8 Pillemer L Schoenberg M D Blum L and Wurz L Properdin system and immunity II Interaction of the properdin system with polysaccharides Science *122* 545 549 1955
9 Frank E Fine J and Pillemer L Serum properdin levels in hemorrhagic shock Proc Soc Exp Biol N Y *89* 223 225 1955
10 Rowley D Stimulation of natural immunity to Escherichia coli infections Observations on mice Lancet Lond *1* 232 234 1955

ENZYMES IN THE HEALING WOUND*

EDWARD L HOWES CONSTANCE M ARMITAGE AND INES MANDL

At a time when enzymes from external sources are being given parenterally and also being added locally to the healing wound an evaluation of what enzymes are already present seems to be appropriate Motivation of healing occurs through enzymatic activity Liquefaction of injured cells takes place by means of proteolytic enzymes found in the secretions and exudate recovered during the exudative phase Sprouting of new capillaries proliferation and maturation of fibroblasts and epithelium ought to be activated by enzymes causing synthesis of nucleic acid and proteins They should be found in granulations and growing epithelium Enzymes occurring in blood and lymph would also be present but whether they would become concentrated about the wound as do inorganic substances has not been determined

Some believe certain pathologic physiologic processes are motivated not

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Amylase was present in serum, lysed cells and pus but was less active in granulations. A small amount of lipase was present in serum and pus but not in granulations. Lysozyme determinations were unsatisfactory because of technical difficulties with the substrate. Granulation dressings failed to produce earlier sprouting of capillaries.

DISCUSSION

The weak character of proteolytic enzymes in the exudate and serum of the rabbit has again been shown. In the human, as has been mentioned, they become strongly positive only in the presence of streptokinase and with disintegration of leucocytes. Streptokinase does not activate rabbit serum and disintegrating leucocytes with a small amount of heat as recommended by Opie did not release them. If they are present, certainly neither lysis of cells nor the formation of pus nor granulations activated them and one would suspect them to become active under these circumstances.

The peptidases found carry proteolytic digestion from the peptides to amino acids. The production of amino acids in the granulations may be for synthesis of proteins or may be a detoxifying mechanism because some peptides are known to be toxic. One can hypothecate that peptides were produced by autolysis but this only means a protein breakdown took place by means of an unmeasurable amount of intracellular proteolytic enzyme. Our finding of the peptidases is in distinct contrast to the work of Barnes[16]. However, we used a more sensitive test, paper chromatography, and a different peptide substrate, leucyl glycine glycine. He tested only with glycyl glycine.

The presence of amylase in pus reported before[16] has not received sufficient attention. The presence of this enzyme does not mean starch is present. However, glycogen is a starch-like substance. Also, we reported elsewhere that mucoproteins concentrate about a wound in granulations[17]. Mucoproteins resist digestion of the usual proteolytic enzymes that dissolve necrotic tissue. Amylase partially attacks them, however. Thus there is built up after the fourth day of healing a protection against proteolytic enzymes about the wounded area, while the amylase present in pus can mobilize mucoproteins. In general then, the wounded area is protected against the external application of proteolytic enzymes except in infrequent instances where proteolytic enzymes that attack mucoproteins are used.

The finding that a small amount of lipase is present recalls that Schilling and Milch[19] recently found lipoproteinase concentrated at the site of injury.

SUMMARY

These studies of enzymes in the healing wounds of rabbits have extended the period of their investigation into that of the formation of granulations. Previous observations on the presence of enzymes in exudative phase have been substantiated. Again the proteolytic enzymes were found to be only weakly positive in the wound of the rabbit. Peptidases were found to be strongly positive in pus and granulations. Their possible roles were discussed. Amylases were also found to be strongly positive in pus and lysed cells but were less active in granulations. A chemical barrier against proteolytic digestion is possibly established by mucoproteins that accumulate about

to determine the amount of starch still present Azocoll is denatured collagen linked to a dye that is released when attacked by trypsin like enzymes[16]

Benzoyl arginine amide is a synthetic substrate from which ammonia is split off by proteolytic enzymes Casein digestion was determined by measuring the decrease in its turbidity[16] Fibrinogen and clotting globulin was incubated with granulations or pus When fibrinolysin was present, the clot liquefied After incubation gelatin remained liquid after cooling if it were digested

Lipase was determined by developing the blue green color of copper soap on starch paste containing butter Lastly peptidases were shown by paper chromatograms After incubation the mixture of peptides and granulation was spotted on paper and developed with phenol sprayed with ninhydrin

RESULTS

The proteolytic enzymes were found to be sparse in serum lysed cells exudate and in the granulations of wounds of the rabbit (See Tables I and 2) Casein benzoyl arginine amide and azocoll were attacked weakly by serum In the presence of infection an enzyme appeared that dissolved gelatin weakly Fibrin was not attacked by serum lysed cells or pus On the other hand peptidases were shown repeatedly in pus and in granulations Peptidases may also be present in serum but the amino acids present masked their appearance as breakdown products

Table 1 Wound — Rabbit — Digestion By

SUBSTRATE	SERUM	LYSED CELLS	PUS (5 DAYS)
Starch	4+	4+	3+
Casein	+	±	0
Gelatin	+	0	0
Benzoyl Arginine			
Amide	0	0	0
Azocoll	+	0	0
Peptides	±	±	4+
Neutral Fat	+	0	+
Fibrin	0	0	0

Table 2 Wound — Rabbit — Digestion By

SUBSTRATE	SERUM	LYSED CELLS	GRANULATIONS (7 DAYS)
Starch	4+	4+	+
Casein	+	+	0
Gelatin	0	0	0
Benzoyl Arginine			
Amide	+	0	0
Azocoll	+	0	0
Peptides	0	0	4+
Neutral Fat	+	0	0
Fibrin	0	0	0

Amylase was present in serum, lysed cells and pus but was less active in granulations. A small amount of lipase was present in serum and pus but not in granulations. Lysozyme determinations were unsatisfactory because of technical difficulties with the substrate. Granulation dressings failed to produce either sprouting of capillaries.

DISCUSSION

The weak character of proteolytic enzymes in the exudate and serum of the rabbit has again been shown. In the human, as has been mentioned, they become strongly positive only in the presence of streptokinase and with disintegration of leucocytes. Streptokinase does not activate rabbit serum and disintegrating leucocytes with a small amount of heat as recommended by Opie did not release them. If they are present, certainly neither lysis of cells nor the formation of pus nor granulations activated them and one would suspect them to become active under these circumstances.

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the wound and in granulations No anabolic enzymes were found by the method of granulations transfer

REFERENCES

1 Menkin V Dynamics of inflammation New York The MacMillan Co 1940
2 Duthie C S and Chain E A Polypeptide responsible for some of the phenomena of acute inflammation *Brit J Exp Path* 20 417 1939
3 Achalme P Recherches sur la presence des ferments soluable dans le pus Compt rend Soc de biol Li 568 1899
4 Achalme P Recherches sur les Proprietes pathogenes de la Trypsine et pouvoir antitryptique du serum des cobayes neufs et immunises Ann Inst Pasteur Par 15 737 752 1901
5 Muller F and Jochmann C Weitere Ergebnisse unser method zum nachweiss proteolytischer ferment wirkungen 3 Mitt Muench med Wschr 53 2002 04 Liii 1906
6 Opie F Intracellular digestion of trypsin like enzyme Physiol Rev 2 552 1922
7 Barker B I The enzymes of fibrin *J Exp M* 10 343 1908
8 Christensen L R Streptococcal fibrinolysis a proteolytic reaction due to a serum enzyme J Gen Physiol 28 363 1945
9 Tillett W S Enzymatic lysis of fibrin and inflammatory exudates by products of hemolytic streptococci Harvey Lectures Series Springfield Ill Chas C Thomas 1949 1950
10 Barnes J M The enzymes of lymphocytes and polymorphonuclear leucocytes Brit J Exp Path 21 264 1940
11 Zamecnik I Stephenson M and Cope O Peptidase activity of lymph and serum after burns *J Biol Chem* 158 135 1945
12 Flemming A Lysozyme a remarkable bacteriolytic element found in tissues and secretions Proc R Soc M Lond 93B 306 17 1922
13 Pruddin J F Lane N and Meyer K Lysozyme content of granulation tissue Proc Soc Exp Biol N Y 72 38 1949
14 Hiatt R P Engle C Flood C and Karush A Role of granulocyte as source of lysozyme J Clin Invest 31 721 1952
15 Selye H Effect upon inflammation of topical treatment with trypsin Proc Soc Exp Biol N Y 86 6 1954
16 Mandl I MacLennan J D and Howes E L Isolation and characterization of proteinase and collagenase from Cl histolyticum J Clin Invest 32 1323 1329 1953
17 Unpublished data
18 Unpublished data
19 Schilling J A and Mich I F Fractional analysis of experimental wound fluid Proc Soc Exp Biol N Y 89 189 1955

RESPIRATORY CARRIER RATES OF *STAPHYLOCOCCUS AUREUS* AND OTHER POTENTIAL PATHOGENS IN SURGICAL PERSONNEL*

JEROME J. LANDY, ROSS S. BENHAM AND ISABELLE HAVENS

By 1952 the respiratory carrier rate of antibiotic resistant *Staphylococcus aureus* (*Micrococcus pyogenes* var. *aureus*) was high in hospital personnel, exceeding 80 per cent in some instances.[2] These carrier rates have been shown to bear a relationship to the infection of "clean" surgical wounds.[3] The current rates and those of 3 years ago for *Staphylococcus aureus* and other potential pathogens in the respiratory tract of surgical personnel and in infected "clean" surgical wounds are compared and contrasted in this study.

METHOD

Three hundred and eighteen cultures from the upper respiratory tracts of personnel of the Department of Surgery, the University of Chicago, taken during the third week of May, 1952, and during the first, second and third weeks of May, 1955, were examined for potentially pathogenic bacteria. Cultures from "clean" surgical wounds which became infected were examined bacteriologically as they were received in the Clinical Microbiology Laboratories of the University of Chicago Clinics. Wounds from June 1, 1951 to May 31, 1952, and from June 1, 1954 to May 31, 1955, referred to respectively as wounds 1952 and wounds 1955, were used for this study.

The potentially pathogenic bacteria from both sources were isolated, identified and tested for sensitivity to antibiotics. Sensitivity of the organisms was determined with each of 8 antibiotics unless otherwise specified (see Table 2, notes). All determinations were made using the paper disk technique. Many of the earlier determinations were confirmed using the tube dilution technique. Since correlation was excellent[4] only the paper disk method was continued.

The results from each year were tabulated and compared. The results from 1952 and 1955 respiratory cultures were used to indicate the trend of long term respiratory carrier rates, and those from 3 successive weekly groups in 1955 were used to indicate short term carrier trends.

For purposes of this paper the organisms studied were grouped either as hemolytic, plasma clumping positive *Staphylococcus aureus*, as enterics (members of the families *Enterobacteriaceae* and *Pseudomonadaceae*), or as other organisms (including *Diplococcus pneumoniae*, *Streptococcus fecalis*, *Streptococcus pyogenes* and *Hemophilus influenzae*).

RESULTS

An inverse relationship existed between the presence of enterics and of antibiotic resistant *Staphylococcus aureus* in respiratory tracts and in wounds during both years of this study. Of respiratory tracts of surgeons (Fig. 1), 42 per cent harbored resistant *Staphylococcus aureus* in 1952, 10 per cent in 1955, and 6 per cent harbored enterics in 1952, 16 per cent in 1955. The analogous figures for wounds are: resistant *Staphylococcus aureus*, 70

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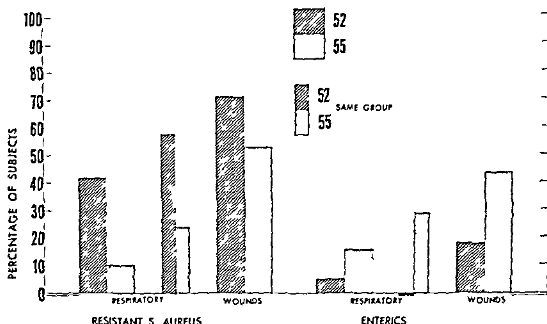


Fig 1 Resistant *S aureus* from respiratory tracts of surgical personnel and from clean surgical wounds compared in 1952 and in 1955 and enteric organisms similarly compared The phrase same group on the chart refers to the fact that the indicated representations are derived from identical subjects studied in both years

per cent in 1952 53 per cent in 1955 and enterics 18 per cent in 1952 44 per cent in 1955 The coefficient of correlation relating the resistant *Staphylococcus aureus* and the enterics is −0 8 for respiratory tracts of surgeons and −0 15 for the wounds Considering that the respiratory studies represent a small number of points in time and that each wound series was collected during an interval of a year these coefficients are in good agreement

Of the surgeons examined in 1952 12 were restudied in 1955 (Fig 1, same group) The per cent of resistant *Staphylococcus aureus* and of enterics in their respiratory tracts in these 2 years showed a change similar to that of the entire group of surgeons and that of wounds

The resistant *Staphylococcus aureus* and enterics occurred together in

Table 1 A Partial Comparison of the Kinds of Organisms Found in the Respiratory Tracts and in the Wounds Studied in 1952 and in 1955

PER CENT OF PERSONS HARBORING	RESPIRATORY TRACTS 1952	RESPIRATORY TRACTS 1955	WOUNDS 1952	WOUNDS 1955
Resistant *S aureus*	4[illegible]	10	70	53
Resistant *S aureus* only	10	4	40	28
Resistant *S aureus* (> one strain)	3	0	2	0
Enterics	6	16	18	44
Enterics only	3	5	9	14
Enterics (>one strain)	3	2	2	9
Resistant *S aureus* + one enteric	0	0	3	12
Resistant *S aureus* +> one enteric	0	2	2	3
Other organisms	23	17	2	16
Other organisms only	6	1	0	1
Resistant *S aureus* + other	8	1	2	6
Resistant *S aureaus* + other + enteric	3	0	0	1
Enteric + other	0	0	0	4

wounds and in respiratory tracts with some frequency (Table 1) The statistical probability of a chance coexistence of the 2 organisms in the same respiratory tract was 0.025 in 1952 and 0.016 in 1955 while the actual rate of occurrence was 0.03 in each of these years In wounds the probability in 1952 was 0.133 and in 1955 it was 0.22 while the actual rates were 0.05 in 1952 and 0.17 in 1955 For the respiratory tracts the ratio of the actual occurrences to the probable was 1.2 in 1952 and 1.9 in 1955 for the wounds the ratio was 0.38 in 1952 and 0.77 in 1955 The proportion of these ratios for the wounds compared to those for the respiratory tracts was 0.32 in 1952 and 0.25 in 1955 This would appear to indicate that there is no mathematically discoverable synergism or antagonism between resistant *Staphylococcus aureus* and enterics in the same wound or respiratory tract

In addition to surgeons whose respiratory tracts were examined in each year a group of auxiliary operating room personnel (scrub nurses etc) who had little contact with patients in clinics and wards was examined in 1955 This group harbored fewer resistant *Staphylococcus aureus* enterics and other organisms than did the surgeons which probably reflects the increased exposure of the surgeons

No respiratory tracts examined in 1955 harbored more than 1 strain of resistant *Staphylococcus aureus* while 8 per cent of those examined in 1952 harbored more than 1 such strain In a number of instances wounds yielded resistant *Staphylococcus aureus* which could be related to the respiratory tract of a specific member of a surgical team [3]

The per cent of resistant *Staphylococcus aureus* in the respiratory tracts of surgeons examined at weekly intervals in 1955 (Fig 2) showed no significant change during 3 weeks Approximately as many individuals lost a strain of resistant *Staphylococcus aureus* as acquired a strain The per cent of enterics varied more

The major antibiotic pattern of *Staphylococcus aureus* in wounds 15 per cent in 1952 and 65 per cent in 1955 is resistance to all antibiotics except chloramphenicol erythromycin and carbomycin or some combination of these (Table 2) This pattern almost disappeared from respiratory tracts

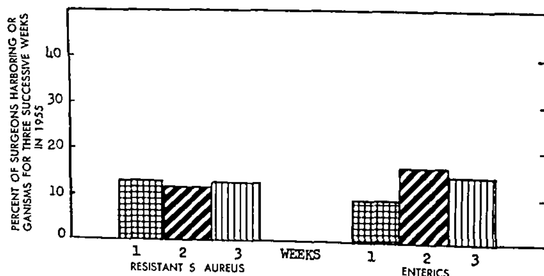


Fig 2 The results of the study of three groups of surgical personnel at weekly intervals during May 1955

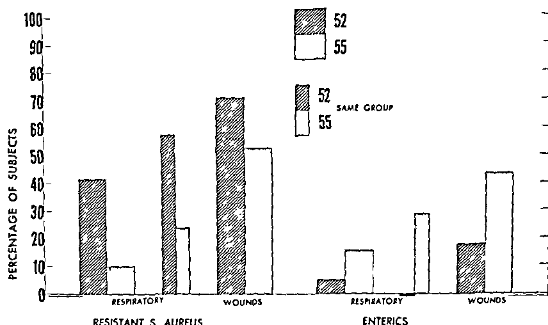


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Table 1 A Partial Comparison of the Kinds of Organisms Found in the Respiratory Tracts and in the Wounds Studied in 1952 and in 1955

PER CENT OF PERSONS HARBORING	RESPIRATORY TRACTS 1952	RESPIRATORY TRACTS 1955	WOUNDS 1952	WOUNDS 1955
Resistant *S aureus*	42	10	70	53
Resistant *S aureus* only	10	4	40	28
Resistant *S aureus* (>one strain)	3	0	2	0
Enterics	6	16	18	44
Enterics only	3	5	9	14
Enterics (>one strain)	3	2	2	9
Resistant *S aureus* + one enteric	0	0	3	12
Resistant *S aureus* +> one enteric	0	2	2	3
Other organisms	29	17	2	16
Other organisms only	6	1	0	1
Resistant *S aureus* + other	8	1	2	6
Resistant *S aureaus* + other + enteric	3	0	0	1
Enteric + other	0	0	0	4

develop appears to be related to the amount of antibiotic generally used. The ability of newly developed resistant strains to become firmly established in respiratory tracts is in part dependent upon their ability to compete in the complex respiratory ecology, particularly in terms of metabolic rates and efficiency.

The work of Lepper *et al.* showed that in the absence of reinfection the incidence of resistant *Staphylococcus aureus* in healthy persons rapidly returned to that found in the general population.[2] This suggests that either an immunologic or a metabolic factor acted to remove the infectious agent. If the factor were immunologic, hospital personnel would probably not have been so heavily infected for so long a period as was the case. If the factor were in the metabolism of the organism, the only requisite for maintaining high infection levels in hospital personnel is a high rate of exposure. Metabolic and growth rates of at least some antibiotic resistant bacteria are less advantageous to the organism than are those of sensitive forms. Therefore the strong inference is that only high rates of development of resistance to antibiotics by bacteria and high exposure rates of hospital personnel have maintained antibiotic resistant *Staphylococcus aureus* as a problem in surgery. Possibly because the more adequate therapy currently used eliminates the sensitive strains from which the resistant strains may develop, the metabolic disadvantages inherent in antibiotic resistance, perhaps aided by some presently unrecognized immune phenomenon in the host, are responsible for the decreasing incidence of infection with antibiotic resistant *Staphylococcus aureus*.

CONCLUSION

Since 1952 there has been a marked decrease in resistant *Staphylococcus aureus* in the respiratory tracts of surgical personnel and some decrease in these organisms in wounds. The number of enteric organisms in both locations has increased in proportion to the decrease of *Staphylococcus aureus*. Surgeons tend to harbor more resistant *Staphylococcus aureus* and other pathogens in their respiratory tracts than do other surgical personnel. Sensitivity patterns and sensitivity to individual antibiotics of *Staphylococcus aureus* have shown changes in the past 8 years.

REFERENCES

1. Benham, R. S., Havens, I., and Landy, J. J.: *Micrococcus Pyogenes* from Surgical Wounds. Bact. Proc., p. 68, May 1954.
2. Lepper, M. H., Dowling, H. F., Jackson, G. G., and Hirsch, M. M.: Epidemiology of Penicillin and Aureomycin Resistant Staphylococci in a Hospital Population. Arch. Int. M. 92:40-50, 1953.
3. Landy, J. J., Havens, I., Clarke, J. S., and Benham, R. S.: An outbreak of wound infections due to antibiotic resistant *staphylococcus aureus*. In Surgical Forum, 1954. Philadelphia, W. B. Saunders Co., 19 , pp. 811-17.
4. Benham, R. S., Havens, I., and Horowitz, M.: Antibiotic sensitivity patterns of *micrococcus pyogenes*. Bact. Proc., p. 68, May 1954.
5. Welch, H.: The antibiotic resistant staphylococci. Editorial. Antibiotics 3:561-70, 1953.
6. Benham, R. S.: Unpublished work.

Table 2 Antibiotic Sensitivities of Staphylococcus Aureus

PER CENT OF *S Aureus* SENSITIVE TO	RESPIRATORY TRACTS 1952*	1955	WOUNDS 1952†	1955
Individual Antibiotics				
Penicillin	33	87	16	21
Streptomycin	68	96	14	26
Chloramphenicol	89	98	86	96
Tetracycline	79	91	x	31
Chlortetracycline	75	91	46	32
Oxytetracycline	79	92	16	31
Erythromycin	100	96	x	84
Carbomycin	100	98	x	96
Antibiotic Patterns‡				
P S C T E Ca	25	81	6	17
– S C T E Ca	39	11	8	8
P – C T E Ca	—	1	1	4
P S C – E Ca	—	5	—	—
– – C T E Ca	7	—	25	2
– – C – E Ca	14	—	}	48
– – C – – Ca	—	1	35	14
– – C – E –	—	—	}	2
– – – – E Ca	7	—	10	1
Others	8	1	8	4

*The cultures represented by the figures in this column were preserved under sterile mineral oil and tested with newer antibiotics as these became available

†At the time these cultures were tested tetracycline erythromycin and carbomycin were not in use Hence these results are based on only 5 antibiotics but are included under the similar patterns among the 8 antibiotics for comparison

‡P = penicillin S = streptomycin C = chloramphenicol T = tetracyclines (tetracycline oxytetracycline chlortetracycline) E = erythromycin Ca = carbomycin A minus sign (–) in the list of antibiotics indicates a lack of sensitivity to that antibiotic Thus the first line reports the percentage of *S aureus* sensitive to all antibiotics tested and the second line those resistant to penicillin only

in 1955 although probable precursors chiefly the *Staphylococcus aureus* resistant to the tetracyclines were present in 6 per cent *Staphylococcus aureus* resistant only to penicillin occurred in 39 per cent of respiratory tracts in 1952 in 11 per cent in 1955 In 1952 25 per cent of *Staphylococcus aureus* in wounds were resistant only to penicillin and streptomycin 2 per cent in 1955

The frequency with which *Staphylococcus aureus* in the respiratory tracts was sensitive to the individual antibiotics increased between 1952 and 1955 in most instances particularly to penicillin and to streptomycin (Table 2) In wounds frequency of sensitivity of *Staphylococcus aureus* to all antibiotics except the tetracyclines increased (but see footnote †, Table 2) In 1955 96 per cent of strains of *Staphylococcus aureus* in wounds were sensitive to chloramphenicol and carbomycin and 84 per cent to erythromycin

DISCUSSION

The recent increase of enteric organisms in wounds and in respiratory tracts of surgical personnel suggests a similarity to the cycle of increase of resistant *Staphylococcus aureus* prior to 1952 followed by the decrease reported in this study

The rate at which antibiotic resistant strains of *Staphylococcus aureus*

Table 1 Representative Tests Showing the Effects of Burns Upon Surface Bacterial Counts

BURN	LOCAL TREATMENT	SIZE OF BACTERIAL FLORA PER SQ CM OF BURNED AREA (× 1000)				
		CONTROL	IMMEDIATELY AFTER BURN	1 DAY LATER	2 DAYS LATER	3 DAYS LATER
Steam 10	Exposed	10.0	0.9	290	0	6160
Steam 30	Exposed	1900	1.7	292000		1190
Steam 5	Dry gauze dressing	3.7	0.56	1.80		
Steam [illegible]	Dry gauze dressing	0.83	0.29	3.7	238	
Steam [illegible]	Vaseline gauze	37.0	3.6	88.00		
Steam [illegible]	Vaseline gauze	0.83	0.29	20	13	1000
Flash 009	Exposed	0.3	1.0	17.6	109	
Flash 009	Vaseline gauze	0.31	0.3	7.8	4,360	

many hundreds of sections of various types of burns at all stages from the immediate post burn period through the stage of ulceration to complete repair showed that countless bacteria could be demonstrated on the skin surface that masses of bacteria could be found in some of the minute epidermal vesicles that few if any bacteria were to be seen in intracellular spaces or in migrating phagocytes in the dermis or subcutaneous tissue but that large numbers of micro-organisms were regularly found in subcutaneous lymphatics and not infrequently in subcutaneous veins These intravascular bacteria were observed in the sub burn area as early as 6 hours after receipt of the injury even in cases in which the heat damaged epidermis had remained unbroken The numbers of bacteria seen in these lymphatic and venous channels appeared in even larger numbers during subsequent days especially after ulceration had occurred until in some instances the vessels were fairly packed with them In older healing wounds the numbers of bacteria seen in the underlying vessels were often diminished and in some instances after 2 3 or 4 weeks when the wounds were healing by extensive fibrosis and also in milder burns where the skin was not completely destroyed but had healed by regeneration no bacteria at all could be found in the underlying vessels

We were naturally skeptical at first as to whether the objects seen in the lymphatics and venules were actually bacteria We questioned whether they might have been only precipitated protein granules fragmented nuclei or artefacts from fixation and staining So the slides were submitted to some equally skeptical pathologists and bacteriologists After study all observers became convinced that the objects in question were in fact bacteria Indeed with experience one learned in examining these sections to distinguish readily between bacteria and artefacts

The question then arose whether these bacteria so characteristically seen in the sub burn lymphatic and venous channels represented dead bacterial cells or live potentially infectious micro organisms which might be carried into the general circulation Attempts to culture these sub burn areas or fluid aspirated from those areas were not successful since we could never be sure that positive cultures were not due to contamination from the skin or negative cultures to ineffective sampling So we placed specific identi

BACTERIAL INVASION IN EXPERIMENTAL BURNS*

PHILIP B PRICE C REED BROWN, THOMAS C KING ROBERTA C PEEK AND LARUE HINCKLEY

This is a brief report of a 4 year bacteriological study of experimental burn wounds

OBSERVATIONS

The bacterial flora of canine skin simulates that of human skin in many respects The transient flora is extremely variable and unpredictable, the resident flora varies in size from animal to animal is larger as a rule than the corresponding flora of humans and the predominating organism is a coagulase negative *Staphylococcus albus* Anaerobes and streptococci are found rarely in the resident cutaneous flora of dogs.

Circular burns 4 cm in diameter were produced on the abdomens or backs of anesthetized dogs Six types of burn were used dry metal contact burns at 70° C steam scalds flame burns flash burns from exploding gasoline vapor high temperature radiant burns and infra red burns Duration of burn target distance and other variable factors were standardized By methods previously described the immediate and delayed effects of heat upon the cutaneous bacterial flora were studied quantitatively and qualitatively

The immediate effect was to reduce the bacterial count of the burned surface least in case of gasoline explosion burns most in the steam scalds but in many hundreds of burns tested there was no instance of complete sterilization of the cutaneous surface Following the injury in all 6 types of burn multiplication of the remaining bacteria occurred so that after 2 to 4 days the bacterial counts per unit surface area far exceed the original control counts As indicated in Table 1 these increases were enormous so that in time the post burn flora was 100 or 1000 or even 10 000 times the size of the pre burn flora This remarkable bacterial increase occurred irrespective of type of dressing used or local application of antiseptic ointments

Early and late effects of burns upon the cutaneous surface flora have been studied qualitatively also This proved to be a long tedious investigation but the results can be stated briefly Although initially application of heat reduced the flora quantitatively it did not alter significantly the flora qualitatively All varieties of surviving bacteria appeared to participate in the post burn increase After the wounds began to break down, there was a tendency of coagulase positive bacteria to increase disproportionately so that in time they predominated Anaerobes and streptococci were encountered rarely

In flash burns healing without any ulceration the cutaneous flora returned to normal size after about 10 days.

In our hands the most satisfactory and reliable method of studying invasion of bacteria into the sub burn tissues was by means of histologic sections stained with ordinary and bacterial stains Careful examination of

*From the Experimental Surgical Laboratory Department of Surgery University of Utah College of Medicine Salt Lake City Utah This study is a joint undertaking of the University of Utah and the Office of Naval Research under Contract Nour 726 (00)

Table I Representative Tests Showing the Effects of Burns Upon Surface Bacterial Counts

BURN	LOCAL TREATMENT	SIZE OF BACTERIAL FLORA PER SQ. CM. OF BURNED AREA (x 1000)				
		CONTROL	IMMEDIATELY AFTER BURN	1 DAY LATER	2 DAYS LATER	3 DAYS LATER
Steam 10	Exposed	10.0	0.9	290	0	.0600
Steam 30	Exposed	[illegible]	1.7	29,200.0		119.0
Steam 5	Dry gauze dressing	3.7	0.36	1.80		
Steam 5	Dry gauze dressing	0.65	0.29	3.7	238	
Steam 5*	Vaseline gauze	57.0	3.6	88.00		
Steam 5	Vaseline gauze	0.65	0.29	20	14	1.000
Flash .009	Exposed	0.5	1.0	17.6	10.9	
Flash .009	Vaseline gauze	0.5	0.3	7.8	4.90	

many hundreds of sections of various types of burns at all stages from the immediate post burn period through the stage of ulceration to complete repair showed that countless bacteria could be demonstrated on the skin surface, that masses of bacteria could be found in some of the minute epidermal vesicles, that few if any bacteria were to be seen in intracellular spaces or in migrating phagocytes in the dermis or subcutaneous tissue, but that large numbers of micro-organisms were regularly found in subcutaneous lymphatics and not infrequently in subcutaneous veins. These intravascular bacteria were observed in the sub burn area as early as 6 hours after receipt of the injury, even in cases in which the heat-damaged epidermis had remained unbroken. The numbers of bacteria seen in these lymphatic and venous channels appeared in even larger numbers during subsequent days, especially after ulceration had occurred, until in some instances the vessels were fairly packed with them. In older healing wounds the numbers of bacteria seen in the underlying vessels were often diminished, and in some instances after 2, 3 or 4 weeks, when the wounds were healing by extensive fibrosis, and also in milder burns where the skin was not completely destroyed but had healed by regeneration, no bacteria at all could be found in the underlying vessels.

We were naturally skeptical at first as to whether the objects seen in the lymphatics and venules were actually bacteria. We questioned whether they might have been only precipitated protein granules, fragmented nuclei or artefacts from fixation and staining. So the slides were submitted to some equally skeptical pathologists and bacteriologists. After study all observers became convinced that the objects in question were in fact bacteria. Indeed with experience one learned in examining these sections to distinguish readily between bacteria and artefacts.

The question then arose whether these bacteria so characteristically seen in the sub burn lymphatic and venous channels represented dead bacterial cells or live, potentially infectious micro organisms which might be carried into the general circulation. Attempts to culture these sub burn areas or fluid aspirated from those areas were not successful, since we could never be sure that positive cultures were not due to contamination from the skin or negative cultures to ineffective sampling. So we placed specific identi

fiable test bacteria on the surface of the wound and after various intervals of time removed the regional lymph nodes under aseptic conditions for culture The test organisms employed were (1) *Serratia marcescens*, a bacterium which is not found on the skin or in the body under normal conditions and which produces a characteristic red pigment in cultures and (2) a phage specific staphylococcus We were able to culture these test bacteria from regional lymph nodes within 8 hours of the time they were placed on the burned surface which provided definite evidence that they had been carried in a live state through the lymphatics

We observed as have many other students of experimental and clinical burns that blood cultures usually remain sterile even after extensive and severe burns until terminal stages when overwhelming perhaps fatal septicemia may make its appearance We postulated that bacteria reaching the sub burn area are carried by lymphatic and venous channels into the general circulation that this occurs in sufficiently small numbers so that the invaders are disposed of promptly by the reticuloendothelial cells and other phagocytes that the circulating blood is thus able to clear itself of bacteria but that eventually the number of organisms poured into the blood stream may become so large that the body defenses are no longer able to combat them successfully

To test this concept we introduced test bacteria into a vein at measured rates and took blood cultures from the opposite side of the body It was found that if the rate of introduction was less than about 1000 bacteria per second negative blood cultures were obtained even when the instillation of test bacteria was continued for many hours at a stretch but when the rate of introduction exceeded that number positive blood cultures could be obtained It was also found that when fewer than 1000 bacteria per second were introduced positive cultures could sometimes be obtained from spleen liver and lung—organs in which reticuloendothelial cells are particularly abundant

The next step was to culture those organs in the case of animals that had received experimental burns The most satisfactory technique was to take surgical biopsies for the purpose from the spleen under sterile precautions either just before the animal was sacrificed or in some instances repeatedly on successive days without sacrificing the animal Many of these biopsies showed no growth on culture unless they were first incubated for 18 to 24 hours whereupon there often grew out large numbers of a variety of organisms Many of these positive cultures contained large Gram positive rods the like of which was not encountered on the surface of the burn wounds We were left uncertain therefore as to the source of these bacteria For all we knew they might have come from the intestinal tract or some break in the skin elsewhere or from the mouth or throat or they may even have been harbored in the normal spleen

The final step in this investigation therefore was to place our specific test bacteria on the burned surface and see whether after due lapse of time they could be recovered from the spleen In many of our animals we were not able to recover these test bacteria but in a small proportion of the animals it was possible to do so and that we feel is a very significant fact It is possible that a larger percentage of positive results might have been obtained had we employed larger wounds

COMMENT

It is assumed that the poorly understood factors which in health regulate the size of the resident cutaneous flora were affected by high temperatures in such a way as to permit excessive multiplication of microorganisms on the skin surface After flash burns which did not destroy the skin this regulating mechanism appeared to be re established in about 10 days

The extraordinary multiplication of microbes on the surface of burned skin and the breakdown of the epidermal barrier evidently set the stage for bacterial invasion of underlying tissues

Our interpretation of the findings detailed in the foregoing section is somewhat as follows epidermis damaged by heat loses its effectiveness as a barrier to bacterial invasion even before it loses its integrity as a physical structure Surface microbes pass more or less readily through the injured or killed epidermis into the dermis where they are picked up by lymph and blood capillaries and are carried to underlying lymphatic and venous plexuses of the subcutaneous space thence they are borne into the general circulation At the outset these live bacteria are discharged into the general circulation at a sufficiently low rate so that with assistance from the reticulo-endothelial system and other body defenses the blood stream is able to keep itself relatively clear of them although live bacteria may occasionally be found in the spleen liver and lung Terminally especially in case of extensive burns and broken epidermis invasion of the blood stream may be so overwhelming that natural defenses are unable to cope with the situation and septicemia ensues

As has been mentioned we were not able to prevent the characteristic increase of surface bacteria by any methods of local treatment Our experimental burn wounds have been exposed to air covered with dry sterile gauze or impervious dressings washed with hexachlorophene soap treated with antiseptics and covered with vaseline gauze or a variety of antiseptic ointments but none of these procedures prevented increase of the cutaneous bacterial flora or penetration of those organisms into the body

The effect of systemic administration of antibiotics is now under investigation Results thus far indicate that they do not prevent surface multiplication but do inhibit invasion *to some extent*

SUMMARY

Small standardized burns in dogs have been studied bacteriologically Even severe burns do *not sterilize the skin* Remaining bacteria multiply on the surface of the burned areas irrespective of local treatment until huge numbers are present After ulceration *occurs* pathogens may predominate Even before ulceration however surface bacteria are able to penetrate the heat damaged epidermis whereupon they are taken up by lymph and blood capillaries and are carried *to the lymphatic* and venous plexuses of the subcutaneous area Histologic sections *properly* stained show them to be present in those vessels in l*arge numbers* Thence they are carried into the general circulation Apparently *the natural* body defenses are able to keep the blood stream virtually *sterile unless* the invasion is overwhelming If that occurs septicemia ensues

Studies on Thermal Burns

THERMAL BURNS IN MAN X SOME PHYSIOLOGIC STUDIES*

JAMES D HARDY

While it is necessary to give large amounts of salt and water to prevent shock in extensive thermal burns there is evidence that such therapy can be excessive It was the purpose of this study to re examine the effects of the infused fluids upon body hemodynamics and fluid equilibrium Measurements of body weight changes cardiac output body fluid compartments insensible fluid loss and the changes in plasma electrolyte concentrations and urine volume were made

Body Weight Changes During Early Fluid Therapy It was assumed that day to day weight changes would be due principally to variations in body water content Thus if the urine volume and the insensible fluid loss were not great the patient would gain weight If losses through the burn wounds were excessive the individual might not gain weight and might actually lose weight depending upon the fluid intake

Fourteen patients whose burns exceeded 15 per cent of the body surface area were studied They were weighed immediately upon admission to the hospital and thereafter at frequent intervals Wherever possible the exposure method was employed so as to avoid error due to dressings saturated with serum When the closed method was employed the patients were weighed on days when the dressings were removed Fluid therapy was administered according to the formula of Evans [1]

Without exception all patients gained weight during early fluid therapy and those whose burns exceeded 40 per cent gained from 5 to 10 kg Subjects whose burns covered 40 per cent of the body surface area received approximately 7 to 8 liters of fluid during the first 24 hours and approximately one half this amount during the second 24 hours Thus it was not surprising that the patients did gain weight during the period when renal function was diminished by hormonal and other factors

Cardiac Output During Early Therapy Cardiac output was measured at the time of admission in 11 burned patients whose injuries exceeded 15 per cent of body surface area and the measurements were repeated almost daily for several days It was assumed that a normal level of cardiac output would reflect a reasonably adequate circulating blood volume It was further assumed that if the purpose of the early fluid therapy was to combat shock by sustaining an effective circulating blood volume then the maintenance of a satisfactory level of cardiac output should reflect

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reasonably adequate fluid therapy regardless of what the current rate of urine formation might be. It was hypothesized that if an adequate cardiac output reflected an adequate state of hydration, then the administration of the minimum amount of fluid required to maintain a normal cardiac output might diminish the risk of overtreatment, a matter of particular concern in the elderly individual.

Cardiac output was variously measured by means of the ballistocardiograph,[5] blue dye,[6,7] and direct Fick methods, though the vast majority of the measurements were made with the dye technique.

On admission the level of cardiac output tended to be within usually accepted normal limits or subnormal before therapy had been begun. After the initial low level, the cardiac output in the extensively burned individual usually rose progressively during the first few days of therapy, in two instances to such markedly elevated levels as approximately 15 liters per minute. This increase in cardiac output was usually associated with an increasing rate of urine flow. Those patients who had been burned for a number of hours before entering the hospital and who had received no therapy tended to have a cardiac output that was less than 5 liters per minute, and 1 patient had the remarkedly low output of approximately 2 liters per minute while in shock prior to the initiation of intravenous colloid therapy.

When in one instance the cardiac output measurements were continued into convalescence, the elevated levels observed in the subacute burn period declined toward normal. These and related data have been reported in detail elsewhere.[7]

It was noted that oliguria could be associated with a low, normal, or elevated cardiac output. A diminished blood pressure level was usually associated with a low cardiac output. It was concluded that the rate of urine flow is a valuable but not infallible guide to fluid requirements in the burned patient.

Thiocyanate Space Measurements.[8] Sodium thiocyanate was injected for the measurement of extra-cellular space. In 7 patients so studied the thiocyanate space increased sharply during early therapy, and this was in agreement with the findings of Cope and Moore.[9] The magnitude of the increase was directly proportional to the volume of fluid infused. In some individuals the increase in thiocyanate space was only 2 to 3 liters while in one individual with an extremely extensive burn (85 per cent flame burn) the increase in the thiocyanate space was 12 liters. The fact that not only did the thiocyanate space increase but that the increase in thiocyanate space exceeded the body weight gain during therapy suggested that the infused fluid provided only one segment of the increase in thiocyanate space. It would appear that the additional fluid had come from the intracellular fluid compartment.

Insensible Fluid Loss. By means of a technique previously described[10] the daily insensible fluid loss during the early post-burn period was estimated in 6 patients. It was found that this loss (from skin, burn wounds and lungs) was usually in the range of from 1 to 3 liters but that it varied considerably from one patient to another and in the same patient from day to day. For example, in one extensively burned subject the loss was approximately 6 liters on 3 successive days. As noted by Cope and

Studies on Thermal Burns

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JAMES D. HARDY

While it is necessary to give large amounts of salt and water to prevent shock in extensive thermal burns, there is evidence that such therapy can be excessive. It was the purpose of this study to re-examine the effects of the infused fluids upon body hemodynamics and fluid equilibrium. Measurements of body weight changes, cardiac output, body fluid compartments, insensible fluid loss, and the changes in plasma electrolyte concentrations and urine volume were made.

Body Weight Changes During Early Fluid Therapy. It was assumed that day-to-day weight changes would be due principally to variations in body water content. Thus, if the urine volume and the insensible fluid loss were not great, the patient would gain weight. If losses through the burn wounds were excessive, the individual might not gain weight and might actually lose weight, depending upon the fluid intake.

Fourteen patients whose burns exceeded 15 per cent of the body surface area were studied. They were weighed immediately upon admission to the hospital and thereafter at frequent intervals. Wherever possible the exposure method was employed so as to avoid error due to dressings saturated with serum. When the closed method was employed the patients were weighed on days when the dressings were removed. Fluid therapy was administered according to the formula of Evans.[1]

Without exception all patients gained weight during early fluid therapy and those whose burns exceeded 10 per cent gained from 5 to 10 kg. Subjects whose burns covered 40 per cent of the body surface area received approximately 7 to 8 liters of fluid during the first 24 hours and approximately one half this amount during the second 24 hours. Thus it was not surprising that the patients did gain weight during the period when renal function was diminished by hormonal and other factors.

Cardiac Output During Early Therapy. Cardiac output was measured at the time of admission in 11 burned patients whose injuries exceeded 15 per cent of body surface area and the measurements were repeated almost daily for several days. It was assumed that a normal level of cardiac output would reflect a reasonably adequate circulating blood volume. It was further assumed that if the purpose of the early fluid therapy was to combat shock by sustaining an effective circulating blood volume, then the maintenance of a satisfactory level of cardiac output should reflect

*Department of Surgery and University Hospital, University of Mississippi Medical Center, Jackson, Mississippi. Based upon studies conducted under Army Contract No. DA 49-007-MD-296 between The University of Tennessee and the Office of the Surgeon General, Department of the Army.

began. Cardiac output was usually normal or subnormal on admission only to rise to levels of between 10 and 12 liters in some of the more extensively burned subjects. In one individual whose study was continued into convalescence the rise in cardiac output gradually subsided to normal levels. Thiocyanate space measurements were made, and it was found that the extracellular fluid volume was markedly augmented during early fluid therapy. Insensible fluid loss varied considerably in different patients and in the same patient from time to time. In one patient the loss during each of three consecutive 24-hour periods was 6 liters. With the occasional exception of carbon dioxide combining power measurements, the values for plasma electrolytes were not of particular aid in the practical management of extensively burned patients.

REFERENCES

1. Evans, E. I., Purnell, O. J., Robinett, P. W., Batchelor, A., and Martin, M.: Fluid and electrolyte requirements in severe burns. Ann. Surg. 135:804, 1952.
2. Hardy, J. D., Jabbour, F., Lovelace, J. R., Neely, W. A., and Wilson, F. C., Jr.: Thermal burns in man. IV. Body weight changes during therapy. Surgery (in press).
3. Starr, I., Rawson, A. J., Schroeder, H. A., and Joseph, N. R.: Studies on the estimation of cardiac output in man and of abnormalities in cardiac function from the heart's recoil and the blood's impacts. The ballistocardiogram. Am. J. Physiol. 127:1, 1939.
4. Hamilton, W. F., Riley, R. L., Attyah, A. M., Cournand, A., Fowell, D. M., Himmelstein, A., Noble, R. P., Remington, J. W., Richards, D. W., Jr., Wheeler, N. C., and Whitman, A. C.: Comparison of the Fick and dye injection methods of measuring cardiac output in man. Am. J. Physiol. 153:309, 1948.
5. Wilson, F. C., Jr., Neely, W. A., and Hardy, J. D.: The blue dye method for the measurement of cardiac output. In Surgical Forum 1953. Philadelphia, W. B. Saunders Co., 1954, p. 88.
6. Cournand, A., and Ranges, H. A.: Catheterization of the right auricle in man. Proc. Soc. Exp. Biol. N. Y. 46:462, 1941.
7. Hardy, J. D., Neely, W. A., Wilson, F. C., Jr., Lovelace, J. R., and Jabbour, F.: Thermal burns in man. V. Cardiac output during early therapy. Surg. Gyn. Obst. 101:94, 1955.
8. Hardy, J. D., Lovelace, J. R., Jabbour, F., and Bramlitt, E. E.: Thermal burns in man. VI. Body fluid compartments during early therapy. Am. Surgeon (in press).
9. Cope, O., and Moore, F. D.: The redistribution of body water and the fluid therapy of the burned patient. Ann. Surg. 126:1010, 1947.
10. Hardy, J. D., Neely, W. A., and Wilson, F. C., Jr.: Thermal burns in man. VII. Insensible fluid loss. Surgery (in press).

Moore[9] the volume of the insensible loss tended to be directly proportional to the extent of the injury

Plasma Electrolyte Changes and Urine Volumes *Plasma Sodium Levels* The plasma sodium concentration did not vary markedly or persistently in the less extensively burned patients In contrast, the 2 most seriously burned subjects both manifested fairly wide fluctuations in these values The admission sodium level of one subject was 163 mEq /l but this level had declined to 131 mEq /l on the fifth post burn day after which it rose and remained at an essentially normal level until death In the second patient the level though normal on admission declined to 116 mEq /l (normal 138 to 141) on the fourth post burn day No effort was made to correct this low level with hypertonic saline solution but it was spontaneously corrected as an increase in urine volume resulted in a decline in body weight

In brief a knowledge of the plasma sodium level did not materially influence the conduct of water and salt therapy

Plasma Chloride Level There was no overall consistent variation in the plasma chloride level As with the plasma sodium level the plasma chloride level was not particularly useful in the practical management of burned patients

Carbon Dioxide Combining Power While there was no consistent variation in these values there was a definitely lowered level in the 2 most severely burned patients and both received sodium lactate intravenously

Plasma Potassium Level Despite a common impression that the plasma potassium level may reach dangerously high levels in the acute burn and significantly low levels in the sub acute and chronic burn we found this value to be generally within normal limits None of the deaths observed was considered to have been due to a derangement in potassium metabolism and virtually no practical therapeutic implications were derived from the many measurements that were performed

The Blood Non protein Nitrogen Level There was almost invariably a rise in this value at some time during the observation of each patient However prognostic implications were not clear so inexact was the relationship between the magnitude of the increase and the extent of the burn

Urine Volumes One simple but important objective of this study was to examine the relationship between the rate of urine flow and the state of body hydration It was concluded that oliguria may be due to inadequate infusion of colloid and/or electrolyte solutions but it may also be a manifestation of the alarm response in the patient whose hydration is adequate In brief the rate of urine flow the systolic blood pressure the plasma CO_2 level the hematocrit and the general clinical evaluation of the patient were all helpful in maintaining an adequate circulatory function while avoiding over treatment

SUMMARY

The effect of early fluid therapy in extensively burned patients has been examined Body weight changes cardiac output changes in extracellular space insensible fluid loss plasma electrolytes and urine volumes have been measured

Extensively burned patients invariably gained several kilograms during the first few days of therapy the weight curve declining as water diuresis

Table 1 Composition of Experimental Diets

DIET NUMBER	PROTEIN SOURCE	COMPOSITION PER 100 GM. DRY WEIGHT: PROTEIN ANALYSIS[1]	PROTEIN CALCULATION[2]	CARBOHYDRATE CALCULATION[2]	FAT CALCULATION[2]
I	Beef	9.3±.96 g	8.[illegible] g	66.[illegible] g	2[illegible].1 g
II	Beef	23.2±1.70	20.4	[illegible]3.4	25.1
III	Beef	40.4±.11	40.2	32.6	25.1
IV	Soya	7.9±1.40	8.4	62.1	25.0
V	Soya	19.7±.30	20.4	39.6	25.1
VI	Soya	39.4±.46	40.0	17.5	18.7
VII	Casein	9.1±.48	8.5	65.5	25.0
VIII	Casein	20.2±.10	20.4	52.8	25.0
IX	Casein	40.8±2.50	40.4	32.2	[illegible]5.0
X	——	0.24±.19	0.5	73.2	25.1

COMPOSITION PER KG. OF MIXTURE[3]: VITAMINS		SALTS	
A	[illegible] U	Ca carbonate	[illegible]
D	[illegible] U	Mg carbonate	[illegible]
Alpha tocopherol	[illegible]0 g	Mg sulfate	[illegible]
Ascorbic acid	[illegible] g	Na chloride	[illegible]
Inositol	[illegible]0 g	K chloride	[illegible]
Choline chloride	[illegible]0 g	K phosphate	[illegible]
Menadione	[illegible] g	Fe phosphate	[illegible]
p-aminobenzoic acid	[illegible]0 g	K iodide	.01
Niacin	[illegible] g	Mn sulfate	.04
Riboflavin	1.0 g	Na fluoride	.01
Pyridoxine hydrochloride	1.0 g	Alk sulfate	.01
Thiamine hydrochloride	1.0 g	Cu sulfate	[illegible]
Calcium pantothenate	[illegible] g		
Biotin	[illegible] mg		
Folic acid	[illegible] mg		
B_{12}	[illegible] mg		

1 By macro Kjeldahl technique on random batches of diet. Factor used was nitrogen x6.2[illegible], except for soya which was nitrogen x5.7
2 From average values of separate ingredients found in standard reference tables
3 Two grams of both mixtures used per 100 grams of diet

THE RELATIONSHIP OF DIETARY PROTEIN TO THE HEALING OF EXPERIMENTAL BURNS*

RICHARD P ANDREWS HARRY C MORGAN AND MAURICE J JURKIEWICZ

A full thickness cutaneous injury provides a wound that is readily observed and measured Consequently it serves admirably as a means of assessing the effects of environmental variables upon the rate of wound healing In view of the relative paucity of data regarding the influence of diet upon the rate of spontaneous healing of third degree burns the first study involved the determination of the effects of feeding no protein and three levels of various native proteins upon the rate of healing of full thickness thermal injuries in rats

METHOD

Adult male Sprague Dawley rats were paired by weights and 1 member of each pair randomly selected to received the burn the other served as a paired control While the rat was anesthetized with ether, the clipped back was exposed to radiant energy from a parabolic reflector Comparable full thickness burns were produced in this way The control members of each pair were anesthetized for a similar period of time but were not burned

Following anesthesia the burned and unburned animals were caged separately and fed the experimental diet The assignment of the diet to each pair was done with the use of a table of random numbers Nine diets containing either casein soya flour or beef and one containing less than 0.5 per cent protein were employed Table 1 gives the chemical composition of these diets

The rate of healing of the wounds was determined by measurements of surface area at intervals of no longer than 10 days The method of Carrel[1] using cellophane to make a tracing of the wound was used The area of the tracing was measured with a planimeter Measurements were performed until healing was complete

RESULTS

All groups contained 6 pairs of animals except the protein free one which consisted of 11 pairs The full thickness burns covered 39.8 ± 7.9 (S D) sq cm or 9.4 ± 1.8 per cent of the body surface of the animals No deaths followed the burns

The measurement of wound size was begun on the tenth day after the injury when accurate delimitation of the full thickness burn was first possible A linear relationship was found to exist between the natural logarithm of the area unhealed and the elapsed time following injury Because this relationship was linear a curve representing the rate of healing could be fitted easily to the data of each group by the method of least squares The slope b of this curve is the measure of the rate of healing of the burns in the group it represents and may be termed the healing rate constant

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III	Beef	40.4±.11	40.2	32.6	25.1
IV	Soya	7.9±1.40	8.4	62.1	25.0
V	Soya	19.7±.30	20.4	39.6	25.1
VI	Soya	39.4±.46	40.0	17.3	18.7
VII	Casein	9.1±.48	8.5	65.3	25.0
VIII	Casein	20.2±.10	20.4	52.8	25.0
IX	Casein	40.8±2.50	40.1	32.2	25.0
X	—	0.24±.19	0.5	73.2	25.1

COMPOSITION PER KG. OF MIXTURE[3]: VITAMINS		SALTS	
A	[illegible] u	Ca carbonate	[illegible]
D	10000 u	Mg carbonate	[illegible]
Alpha tocopherol	[illegible]	Mg sulfate	[illegible]
Ascorbic acid	[illegible]	Na chloride	[illegible]
Inositol	[illegible]	K chloride	[illegible]
Choline chloride	[illegible] g	K phosphate	[illegible]
Menadione	[illegible] g	Fe phosphate	[illegible]
p-aminobenzoic acid	[illegible] g	K iodide	.01
Niacin	[illegible] g	Mn sulfate	.01
Riboflavin	1.0 g	Na fluoride	.01
Pyridoxine hydrochloride	1.0 g	Al K sulfate	.01
Thiamine hydrochloride	1.0 g	Cu sulfate	[illegible]
Calcium pantothenate	3.0 g		
Biotin	20 mg		
Folic acid	[illegible] mg		
B_{12}	1.3 mg		

1 By macro Kjeldahl technique on random batches of diet. Factor used was nitrogen × [illegible] except for soya which was nitrogen × [illegible]
2 From average values of separate ingredients found in standard reference tables
3 Two grams of both mixtures used per 100 grams of diet

The uncorrected as well as corrected values for these constants (or slopes) for the groups of animals eating each diet are presented in Table 2 The correlation coefficient r of each slope serves as an indication of individual variance about the curve Statistical comparison of the slopes of the various curves was effected by the method of Fisher

The corrected slopes were obtained by eliminating from each group the animals whose wounds developed thick cobblestone crusts and exuded large amounts of pus or serum Whenever cobblestone crusts and excessive exudation occurred the rate of healing slowed remarkably (See curves – Fig 1) The above changes occurred in the wounds of some animals in every group excepting the group Diet II No more than 3 animals were affected in any group The effect of correction of all slopes is shown in Table 2 The corrected and uncorrected slopes of groups II and X are graphically demonstrated in Fig 1

DISCUSSION

The experimental data show that the rate of healing of full thickness thermal injuries covering 9 4 ± 1 8 per cent body surface of the rats was not influenced by the amount or the source of protein fed although the amount of protein eaten per diem varied between 05 and 8 gm and the protein sources were as different as casein beef and heated soya flour

Although 2 of the uncorrected slopes vary significantly from all others this variation has no relationship to either the amount or source of protein in the diets For example the healing rate of animals eating Diet II (beef, 23 2 gm per cent was significantly faster than all others yet the groups eating Diets I (beef 9 3 gm per cent) and Diet III (beef 10 2 gm per cent) were well within the mean range Similarly, the slope of the

Table 2 Healing Rate Slopes of Dietary Groups and Their Statistical Comparison

DIET NUMBER	UNCORRECTED SLOPE b	CORRELATION COEFFICIENT r	t	PROBABILITY t	CORRECTED SLOPE b	CORRELATION COEFFICIENT r	t	PROBABILITY t
I	— 02567	— 67	1 03	1	— 04966	— 89	08	1
II	— 05085	— 94	8 12	01	— 05085	— 94	55	1
III	— 02899	— 67	17	1	— 04902	— 87	08	1
IV	— 02590	— 81	97	1	— 04865	— 76	91	1
V	— 03051	— 84	24	1	— 04741	— 91	61	1
VI	— 02121	— 69	2 73	.01	— 04737	— 78	31	1
VII	— 03163	— 85	33	1	— 04872	— 87	18	1
VIII	— 02341	— 74	1 53	1	— 05063	— 89	.29	1
IX	— 02347	— 67	1 75	1	— 05059	— 96	.51	1
X	— 03361	— 74	1 92	05	— 05070	— 86	38	1

t values from the table of Fisher and Yates for the range of probabilities with the number of observations used are

t for P 1 = 1 67 1 70
t for P 05 = 2 00 2 04
t for P 01 = 2 66 2 75

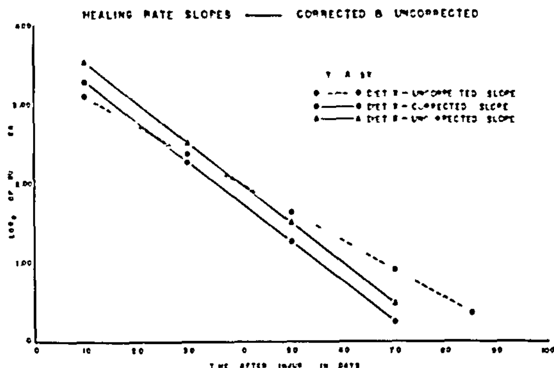


Fig 1

animals eating Diet VI (soya 10.0 gm per cent) was significantly slower but the slopes of animals eating Diets IV (soya 8.1 gm per cent) and V (soya 20.1 gm per cent) were grouped within limits of the mean

Correction of the slopes by excluding animals developing gross changes in their wounds as noted above eliminated this variation in healing rate The corrected slopes were not only statistically similar to each other but also paralleled the slope of the unaffected animals (Diet II) Cobblestone crusting of and exudation from some wounds as well as the accompanying decrease in healing rates are probably related to infection However sufficient data are not available to confirm this

SUMMARY

A study of wound healing in burns was presented The linear relationship between the natural logarithm of burn area and time lapse after injury was used to determine healing rate slopes

Experimental diets varying from 21 to 10 gm per cent protein with either beef casein or soya flour as the sole protein source were found to have no effect on the rate of healing of full thickness burns covering 9.1 ± 1.8 per cent of the dorsal body surface of mature male rats

REFERENCES

1 Carrel A and Hartmann A Cicatrization of wounds J Exp M 24 429-450 1916
2 Fisher R A Statistical methods for research workers London Oliver & Boyd 1941

THE INFLUENCE OF GROWTH HORMONE ON THE NITROGEN BALANCE OF THE SEVERELY BURNED*

JOHN F PRUDDEN, ELINOR PEARSON AND HARRY S SOROFF

The effects of growth hormone on the nitrogen balance of severely burned patients has been studied by means of carefully randomized experimental metabolic periods (patients receiving the hormone) in a background of control periods of identical length during which the preparation was withheld

The subjects for this study were 4 patients with greater than 30 per cent third degree burn 3 males and 1 female A number of mineral balances were analyzed as well as the nitrogen balances As indicated above the spacing of the experimental (growth hormone) *periods was planned so* that there was a randomization of dressings graftings and other special procedures

The results were plotted in the following 4 ways

(1) Nitrogen balance per gram of nitrogen intake versus time
(2) Nitrogen balance versus nitrogen intake

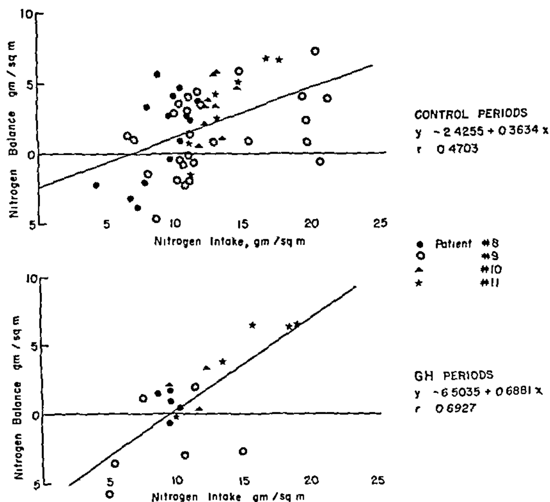


Fig 1

*From the Surgical Research Unit Brooke Army Medical Center Fort Sam Houston Texas

(3) Nitrogen intake minus urine total nitrogen versus nitrogen intake.

(4) Nitrogen intake minus urine urea nitrogen versus nitrogen intake.

In the cases of methods Number 2 through Number 4 the data was normalized by converting it to the square meters of body surface in order that all the patient data could be plotted together. In the first method the individual patients were plotted and analyzed.

In all graphing methods the growth hormone data comprised one plot and the controls another. After fitting of the curves, comparisons were made of the slopes, of the zero intake (or time in method Number 1) intercepts, of the coefficients of correlation, and of the balances at specific intakes in the last 3 techniques.

The most general plot, that of the nitrogen balance in grams per square meter of body surface in relation to the nitrogen intake normalized to the same dimensions, is shown in Figure 1. The better correlation coefficient (r), the steeper slope (the coefficient of x), and the lower y intercept (the value of y when x is zero) which characterize the growth hormone plot are demonstrated. All of these differences are significant with a p value $< .001$.

The results were in general agreement by all methods of calculation. They indicate that growth hormone is an effective stimulator of nitrogen anabolism providing the intake is high (above about 11 grams per square meter of body surface) and providing the individual receiving it is already in spontaneous anabolism. The time plots showed that there was a definite lack of a favorable effect when the patient was in a catabolic situation. It is true, however, that there was also a lack of an adequate nitrogen intake at those times. Therefore the possibility exists that the response to growth hormone would be satisfactory even in catabolism were it possible to provide an adequate intake of nitrogen in the background of a good nutritional program. This matter is under present investigation. The data presented appears to indicate that this is unlikely and that growth hormone requires the presence of other endocrine factors in order to bring about a more effective nitrogen anabolism.

THE EFFECT OF THERMAL INJURY ON INSENSIBLE WEIGHT LOSS IN THE RAT*

Preliminary Report

HARRY C MORGAN, RICHARD P ANDREWS AND MAURICE J JURKIEWICZ

Among the methods for determining the metabolic rates of mammals, the measurement of the rate of insensible loss of weight is a simple yet accurate one[1] The insensible weight loss consists of the losses of carbon dioxide and water vapor through skin and lungs less the mass of oxygen absorbed The total heat produced can be calculated from the insensible loss of weight provided the loss is determined while the respiratory quotient is steady Constancy of the respiratory quotient is readily attained by a preliminary 24 hour fast

This study of the effect of standard burns on the rates of insensible weight loss was undertaken with the hope that more might be learned of the changes in metabolism associated with thermal injuries

METHOD

Eight adult male Sprague Dawley rats were paired by weight Following multiple determinations of insensible weight loss in the fasting state at weekly intervals 1 member of each pair was randomly selected to receive a standard full thickness burn covering approximately 14 per cent of the body surface The other served as a paired control Both animals were anesthetized with ether The clipped back of the selected rat was exposed to radiant energy from a parabolic reflector The animals were caged separately and fed a diet containing 20 gm per cent casein Each pair was fasted for 24 hours prior to every metabolic determination Determinations of insensible weight loss were performed on the burned and unburned control about every 7 days while the burns were healing All animals had access to water during the fasting period

Four additional male rats of similar age and weight were given the same diet and preburn control readings recorded This second group of rats was treated exactly in the same manner except that they were given full thickness burns about one half as large as the first group

The insensible weight loss was determined with chainomatic recording balances The frequency duration and gross magnitude of animal movements were automatically recorded Consequently periods of quiescence would be selected and the rates of insensible weight loss measured during these inactive periods

The rate of healing of the wounds was determined by measuring the surface area of the wound at frequent intervals beginning on the eighth postburn day The method of Carrel using cellophane to trace the perimeter of the wound and measuring the area with a compensating polar planimeter was used These determinations were made until healing was complete

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*Department of Surgery Washington University School of Medicine St Louis Mo

Table I. Abstract of Metabolic Changes Following Burns

WEEKS	RAT NO 3 BURN NON-INFECTED			RAT NO 6 CONTROL		RAT NO 7 BURN INFECTED WORST			RAT NO 8 CONTROL	
	WT	IWL	BURN AREA	WT	IWL	WT	IWL	BURN AREA	WT	IWL
Before Burn	2[illegible]	710±40		[illegible]	[illegible]	[illegible]	[illegible]		2[illegible]0	[illegible]3±3[illegible]
			1[illegible] BS					1[illegible] BS		
After Burn 2	[illegible]72	19[illegible]0	[illegible]2.6	[illegible]0	[illegible]	22[illegible]	14[illegible]0	[illegible]10	2[illegible]1	1[illegible]
8	[illegible]00	100[illegible]	9.2	[illegible]	601	2[illegible]	14[illegible]0	1[illegible]9	318	[illegible]
1[illegible]	[illegible]	[illegible]8	2[illegible]	421	[illegible]	2[illegible]9	11[illegible]	[illegible]0	[illegible]0	[illegible]0
18	[illegible]6	7[illegible]2	healed	4[illegible]0	10[illegible]0 (diarrhea)	[illegible]	1410	3.0 "infected"	3[illegible]	[illegible]
22	331	[illegible]		4[illegible]0	831	[illegible]1	[illegible]	3.2 "crust"	3[illegible]	[illegible]03

IWL = Insensible weight loss mg./2 hours
Burned Area = Expressed as sq. cm. and per cent body surface
Weight = Fasting weight in gm.

RESULTS

The normal rates of insensible weight loss amounted to 636±69 (S.D.) mg. per 2 hours over a 3 week interval prior to burning (11.8±0.8 per cent body surface). The rate of insensible weight loss began to rise within 3 days after the burn and reached peak levels of 280 to 310 per cent of normal by the twentieth to the twenty-eighth postburn day. Thereafter a slow erratic decline toward normal was observed. The rates finally reached normal ranges just before, simultaneously with, or shortly after wound healing was complete—16 to 23+ weeks after injury. The rate of insensible weight loss in the control animals remained constant at 682±160 mg. per 2 hours throughout the 22 weeks. The only exception was rat Number 6 which developed an unexplained rise from the seventeenth to nineteenth week.

The second group of 4 animals with full thickness burns comprising 28.0 ± 1.3 (S.D.) sq. cm. or 8.0 ± 1.1 per cent of the body surface had a control rate of insensible weight loss of 551 ± 95 (S.D.) mg. per 2 hours. The rate of insensible weight loss started to increase within 72 hours after injury reaching peak levels of 190 to 215 per cent of normal in 12 to 17 days. These elevations slowly declined and approached normal levels 11 to 75 days later. The rate of insensible weight loss approached normal as the wounds healed. However, the rate of decline lagged behind the rate of healing. There was no significant difference in caloric intake between the burned and control animals.

DISCUSSION

In every instance following thermal injury the rate of insensible weight loss was much increased This indicates that the metabolic rates rose Although the duration of the rise in insensible weight loss roughly corresponded to the period of gross weight loss in every burned animal the sustained elevations of insensible losses were maintained during the time the burned animal gained rare weight This gain in weight of the burned animals occurred while the insensible weight losses were 62 to 200 per cent above normal and while they ate no more than their paired controls

In 2 instances (rats Number 2 and 7) the elevation of the rate of insensible weight loss was maintained for longer periods of time This correlated with the appearance of a thickened cobblestone crust with purulent exudation probably due to infection although sufficient data to prove this is lacking The rate of healing was markedly slowed during this period of wound crusting

SUMMARY

A study of the effect of thermal injury on the rate of insensible weight loss is presented

In every instance following full thickness burns covering 6 9 to 15 7 per cent of the body surface the rate of insensible weight loss or metabolic rate was elevated and approached normal ranges as the wound healed However the rate of decline lagged behind the rate of healing

After an initial weight loss phase the burned animals gained weight while their rates of insensible weight losses were 62 to 200 per cent above normal although they did not eat significantly more than their paired controls

REFERENCES

1 Greene J A and Luce R J Determination of basal metabolism of the albino rat from the insensible loss of weight J Nutrit 4 371 378 1931

2 Carrel A and Hartman A Cicatrization of wounds J Exp M 24 429 450 1916

LOCAL EDEMA AND CAPILLARY PERMEABILITY ASSOCIATED WITH BURN WOUNDS*

THOMAS C KING LEVI E REYNOLDS AND PHILIP B PRICE

Much of the tissue damage that follows experimental burns is delayed and is disproportionate to the amount of heat actually penetrating the skin Factors responsible for this phenomenon are obscure The present study was undertaken to define more clearly the role of abnormal capillary permeability in these changes

That capillaries become abnormally permeable to the plasma proteins locally following thermal injury has been clearly demonstrated[1,2] As a means of studying the loss of these proteins across capillary membranes we

*From the Experimental Surgical Laboratory Department of Surgery University of Utah College of Medicine Salt Lake City Utah This study is a joint undertaking of the University of Utah and the Office of Naval Research under Contract Nour 726 (00)

have utilized Evans blue dye (T 1824) Even in the concentrations used by us this dye is virtually all bound to plasma proteins,[4] particularly to the albumin fraction as shown by electrophoretic migration characteristics[5] We have assumed therefore that following the injection of the dye into the blood stream abnormal staining of tissues is a true index of abnormal permeability to plasma colloids

METHODS

Observations were made on 4 types of burns in healthy anesthetized mongrel dogs The burns each about the size of a silver dollar were made at various time intervals from 5 minutes up to 7 days prior to the intravenous injection of a solution of 5 per cent T 1824 dye in normal saline A uniform dose of 25 mg of dye per kg of animal weight was employed The types of burns included in this study were (1) low temperature dry metal contact burns roughly analogous to hot water bottle or radiator burns (70 C for 60 seconds duration) (2) steam scald (95° C at the altitude of our laboratory for 5 seconds duration (3) alcohol flame burns which simulate the thermal injury received from burning clothes or houses (about 750 C for 10 seconds duration) and (4) flash burn from explosion of high octane gasoline vapor in oxygen (approx 2000 C 0 009 seconds duration) Apparatus and techniques employed in producing the burns have been previous described[6]

OBSERVATIONS

70 **Dry Contact Burn** This burn results in immediate blanching with superficial heat necrosis of the entire burn area however after a few seconds delay a ring of erythema appears circumscribing the burn During the next 24 hours the edema which initially had been well localized to the area of erythema diffuses widely and soon becomes imperceptible The well defined ring of erythema persists until about the fourth day When T 1824 is injected the dye appears almost immediately in all burns less than 1 hour old but after greater or less delay in older burns (Table 1) The discoloration appears as a deep blue ring extending from the burn margin out about 5 mm to 10 mm and is sharply defined The central burned area remains blanched and at no time is any dye visible there A knife cuts through the burned area with slightly more difficulty than through the surrounding tissues and in the burns under 1 hour old a large amount of thin watery clear blue fluid exudes from the wound While the entire thickness of the dermis of the burned area is free of any grossly detectable dye the skin around the burn margin is deeply stained In burns over 6 hours old there is very little stained free fluid present

Flame Burn The general appearance and development of the alcohol flame burn is similar in some respects to that of the dry burn Significant differences are probably due to the mechanical protection afforded by coagulation and charring during the early seconds of the burn The skin is hard contracted extremely difficult to cut and forms a depression surrounded by a blanched puffy ring of edematous skin This in turn is surrounded by a well defined zone of erythema Staining is lighter and is more sharply limited in extent than in the dry burn The burned skin itself is completely free of dye while surrounding dermis and subcutaneous tissues are deeply stained Older burns (3 to 7 days old) show very little staining

DISCUSSION

In every instance following thermal injury the rate of insensible weight loss was much increased. This indicates that the metabolic rates rose. Although the duration of the rise in insensible weight loss roughly corresponded to the period of gross weight loss in every burned animal, the sustained elevations of insensible losses were maintained during the time the burned animal gained tare weight. This gain in weight of the burned animals occurred while the insensible weight losses were 62 to 200 per cent above normal and while they ate no more than their paired controls.

In 2 instances (rats Number 2 and 7) the elevation of the rate of insensible weight loss was maintained for longer periods of time. This correlated with the appearance of a thickened cobblestone crust with purulent exudation, probably due to infection, although sufficient data to prove this is lacking. The rate of healing was markedly slowed during this period of wound crusting.

SUMMARY

A study of the effect of thermal injury on the rate of insensible weight loss is presented.

In every instance following full thickness burns covering 6.9 to 15.7 per cent of the body surface the rate of insensible weight loss or metabolic rate was elevated and approached normal ranges as the wound healed. However, the rate of decline lagged behind the rate of healing.

After an initial weight loss phase, the burned animals gained weight while their rates of insensible weight losses were 62 to 200 per cent above normal although they did not eat significantly more than their paired controls.

REFERENCES

1. Greene, J. A., and Luce, K. L. Determination of basal metabolism of the albino rat from the insensible loss of weight. J. Nutrit. 4: 371-378, 1931.
2. Carrel, A., and Hartmann, A. Cicatrization of wounds. J. Exp. M. 24: 429-450, 1916.

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THOMAS C. KING, LEVI E. REYNOLDS AND PHILIP B. PRICE

Much of the tissue damage that follows experimental burns is delayed and is disproportionate to the amount of heat actually penetrating the skin. Factors responsible for this phenomenon are obscure. The present study was undertaken to define more clearly the role of abnormal capillary permeability in these changes.

That capillaries become abnormally permeable to the plasma proteins locally following thermal injury has been clearly demonstrated.[1, 2] As a means of studying the loss of these proteins across capillary membranes we

*From the Experimental Surgical Laboratory, Department of Surgery, University of Utah College of Medicine, Salt Lake City, Utah. This study is a joint undertaking of the University of Utah and the Office of Naval Research under Contract Nonr 726(00).

plethysmograph to measure total edema formation following hot water burns and pressure dressings.

Local cooling of the burned area by means of cold compresses applied promptly and continued for 20 to 30 minutes following the injury appeared to control to some extent the escape of plasma proteins into the damaged areas. In 2 second steam burns and in 10 second low temperature dry contact burns, the diffusion of the dye seems to be significantly inhibited and even after termination of the cold compresses, the degree of discoloration and detectable edema never reaches the proportions regularly observed in the uncooled burns.

DISCUSSION

An interesting and perhaps significant observation is that increased capillary permeability and consequent edema does not occur at the area of severe thermal trauma but rather in adjacent areas where presumably the damage is more moderate. Furthermore, the areas of eventual necrosis and sloughing seem to be precisely those areas of the burn which do not show any appreciable dye infiltration either at the time of the burn or subsequently. A possible explanation is that capillaries of the severely traumatized portion are not only damaged themselves but are also bereft of a dynamic blood supply.

To explain tissue damage following burns, at least 3 factors may be postulated: (1) immediate heat necrosis, which is more superficial and limited as a rule than is generally realized; (2) more gradual loss of adjacent tissues due to ischemic necrosis; and (3) secondary bacterial invasion which results in further cellular damage. Although capillary thrombosis following thermal injury has been described by other workers[7] this has not been the usual finding in histopathological studies in our laboratory. Early progressive bacterial invasion is consistently noted, however.

It might be argued that abnormal permeability at the areas adjacent to the burn site is an effect of heat conducted from the burn proper. Previously reported studies[6] in our laboratory have shown, however, that actual increase in temperature at these areas is too small, in all probability, to account for the outpouring of plasma. Histamine release[4], reflex neural mechanisms, an intriguing "chain reaction" theory of cellular breakdown[8] and numerous other explanations have been suggested but a discussion of them is not within the scope of this paper.

CONCLUSIONS

1. Edema varies in degree depending upon the severity of the burn.
2. Edema formation appears to be closely associated with and roughly proportional to abnormal capillary permeability to plasma proteins tagged with T-1824 dye.
3. About 75 per cent of the total edema forms within the first 15 minutes following burns and 85 per cent to 90 per cent within the first hour. Very little edema is formed after 6 hours.
4. Capillaries remain permeable to a progressively diminishing degree for 2 to 4 days following significant thermal injury.
5. Pressure applied immediately to burns does not appear to prevent or significantly inhibit the escape of plasma proteins but merely displaces the edema formation to the area surrounding the pressure plate.

Table 1 Capillary Permeability, Indicated by T 1824 Dye, Following 70° C Dry Metal Contact Burns of 60 Second Duration

TIME OF INJECTION	ELAPSED TIME BEFORE APPEARANCE OF DYE	EXTENT OF DISCOLORATION MEASURED RADIALLY FR EDGE OF WOUND	DEGREE OF DISCOLORATION IN SURROUNDING EPIDERMIS	DEGREE OF DISCOLORATION IN SUBCUTANEOUS TISSUE	TIME REQUIRED TO REACH FULL COLOR DEPTH
1 hr before burn	<1 min	10 mm	+++++	+++++	15 min
5 min after burn	<1 min	10 mm	+++++	+++++	15 min
1 hr after burn	1 min	10 mm	++++	+++	20 min
6 hr after burn	3 to 4 min	10 mm	++++	+++	1 hr
12 hr after burn	5 min	9 mm	+++	++	1 hr
24 hr after burn	5 to 10 min	8 mm	++	+	1 hr
2 days after burn	12 to 15 min	6 mm	++	+	1 hr
4 days after burn	25 to 30 min	2 mm	+	±	1 hr
7 days after burn	>1 hr	0 mm	±	—	?

and almost no exudate Flame burns show less tendency to breakdown and slough than dry burns

Scald Burn Initially the steam burn does not appear as abnormal as the 2 types just described Edema is more diffuse and apparent tissue damage is less and by the twenty fourth hour it is often difficult to distinguish the burn area from normal skin However, injection of the dye reveals that a severe degree of damage has actually occurred for the tissues are deeply stained over a wide area and there is much more free deeply stained fluid than in the other types of burns In steam burns 1 to 7 days old injection of dye produces very faint staining which extends only to the most superficial subcutaneous layers Nevertheless it is evident by this time that more tissue destruction has taken place in steam burns than in the other types and a deep slough appears to be impending over a wide area

Flash Burn In spite of the high temperatures reached in the gasoline oxygen explosion burns the damage done to tissues seems to be relatively small There is no appreciable edema and no dye could be detected in any of the burns

Other Observations When pressure is applied over a burn it is noted that dye discoloration is displaced in that it develops around the edge of the pressure plate after 3 or 4 minutes delay It soon reaches a color depth approximating that in a similar burn not subjected to the pressure If the pressure is removed within 2 hours following the burn the dye appears around the burned area much as if the pressure had never been applied This finding seems to corroborate Cope's[2] studies in which he utilized the

EFFECTS OF LOCAL CHILLING IN THE TREATMENT OF BURNS*

LYAL I REYNOLDS C REED BROWN AND PHILIP B PRICE

During a study of local edema and capillary permeability in burn wounds it was noted that application of cold to the burned area resulted in significant inhibition of local edema formation and capillary permeability to plasma proteins This observation led to an investigation of local chilling as a method of therapy in this type of injury

LOCAL EFFECTS

Anesthetized close clipped white mongrel dogs were injected intravenously with Evans blue dye (20 mgm /kgm body weight) and circular steam burns were made 1 cm in diameter and of 3 to 5 seconds duration When cold was applied immediately to the burned areas and was continued for 15 to 30 minutes the rate of dye extravasation was seen to be retarded and the total amount of blue color in the tissues definitely decreased The chilling seemed to have less effect when its application was delayed longer than 1 minute although some local benefit could be noted even after 5 minutes delay in that there was less extravasation of edema fluid Water varying in temperature from 1° C to body temperature in increments of 5 degrees was applied to burned areas and it was found that ordinary cold tap water between 10 C and 15 C was optimum for this purpose

MORBIDITY

Next we studied the effects of cooling on morbidity Anesthetized dogs were dipped uniformly in water at 92° C for 15 to 20 seconds Half of these burned animals were then promptly immersed in cold tap water at 15° C for 30 minutes the remaining half were left untreated It was noted that all of the unconscious animals placed in the 92° C water bath showed pronounced involuntary contractions of the body musculature including the larynx The group of animals burned and subsequently immersed in cold tap water at 15° C appeared to relax promptly and became quiet Furthermore, the burned areas in the chilled animals looked definitely less erythematous than in the untreated animals and there was less edema When the treated animals awakened they were friendly alert and seemingly free from pain whereas the uncooled animals appeared sick less friendly and obviously very uncomfortable

Bacteriological studies showed that the chilling did not have any significant effect upon the cutaneous flora of the burned area

MORTALITY

To study the effects of cooling on the mortality of experimentally burned animals C57 black mice and CFW white mice were used Preliminary tests revealed that dipping the caudal half of the mice in water at 70° C for 10 seconds killed approximately 95 per cent of the mice in 24 hours and 100 per cent of the mice in 7 days Three hundred mice were lightly anesthe

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*From the Experimental Surgical Laboratory Department of Surgery University of Utah College of Medicine Salt Lake City Utah This study is a joint undertaking of the University of Utah and the Office of Naval Research under Contract Nonr 726 (00)

6 Prompt cooling of the burned area affords a moderate degree of inhibition of abnormal capillary permeability and reduces excessive loss of blood proteins into the adjacent tissues

SUMMARY

In order to study the role of capillary permeability in the pathogenesis of burn damages, T 1824 (Evans blue) dye was injected into dogs subjected to 4 standardized types of thermal injury low temperature dry contact burn steam scald alcohol flame char burn and exploding gasoline vapor flash burn The dye was injected at various intervals up to 7 days following receipt of the burns A close parallel was found to exist in fresh burns between degree and extent of edema and capillary permeability While the edema formation takes place primarily within the first hour following the burn capillaries remain permeable to a degree for 2 to 4 days The abnormal egress of dye from the capillaries was not effectively controlled by pressure but could be inhibited at least in part by prompt application of cold compresses to the burned area

REFERENCES

1 Drinker *et al* Lymph studies in sterile inflammation J Exp M *56* 363 1932
2 Cope *et al* The nature of the shift of plasma protein to the extravascular space following thermal trauma Ann Surg *128* 1041 1948
3 Price and Longmire Use of T 1824 in plasma volume determinations Bull Johns Hopkins Hosp *71* 51 83 1942
4 LeVeen and Fishman Combination of Evans Blue with plasma protein Its significance in capillary permeability studies Am J Physio *151* 25 1947
5 Rawson Evans Blue and plasma proteins Am J Physio *138* 708 1943
6 Price *et al* Penetration of heat in thermal burns in Surgical Forum 1952 Philadelphia W B Saunders Co 1953 pp 433-438
7 Sevitt Local blood flow changes in experimental burns J Path Bact Lond *61* 427 1949
8 Sevitt The failure of anti histamine drugs to influence the capillary permeability in experimental burns Brit J Exp Path *30* 540 1949
9 Dougherty T E Personal communication
10 Cope *et al* Pressure Bandages in Burns Arch Surg *59* 1059 1949

of repeating these studies in larger animals so as to reduce the effects of generalized hypothermia. These studies are incomplete but so far they seem to show that the cold water treatment effectively reduces the mortality rate of extensive burns even when the body temperature is not greatly reduced.

CLINICAL APPLICATION

Naturally we have been much interested in the feasible application of these observations to the treatment of burns in human beings. So far we have encountered 4 instances of accidental burn injury in humans in which it was possible to apply cold within 1 minute after the burn occurred. One of these burns, caused by boiling grease, was limited to the dorsum of the foot; 2 of these burns were fairly extensive. In all of these patients the application of cold afforded immediate, almost complete relief of pain, and the burns appeared to heal more promptly and with less tissue destruction than previous clinical experience had led us to anticipate.

SUMMARY

A study has been made of local chilling in the treatment of experimental burns. It has been found that application of cold water within 1 minute and continued for 15 to 30 minutes is beneficial. Local edema, capillary permeability, erythema, and pain are reduced, and the animals appeared less sick than the untreated burned animals. Twenty-four hour mortality was reduced from 95 per cent in the untreated control group of severely burned mice to 37 per cent in the group treated by chilling.

This method of treatment has been tried in a small number of accidental human burns with encouraging results.

tized with ether and were dipped uniformly in a 70° C water bath up to a level 3.5 cm above the base of the tail, for 10 seconds. Exactly 1 minute after the burn half of the animals were dipped in 15° C tap water for 10 minutes; the remaining half were untreated. Additional control animals that were simply anesthetized and cooled recovered without incident.

The burned and chilled mice upon awakening appeared comfortable and resumed normal activities, whereas the untreated group appeared agitated and crouched in a corner of the cage and would not eat.

Various temperatures of water from 1° C to 37° C were used for the chilling effect. It was found that tap water 10° C to 15° C was optimum and that no additional benefit accrued from using lower temperatures and there seemed to be decreasingly less benefit from higher temperatures.

The mortality of the mice dipped in the manner described in water at 70° C for 10 seconds and left untreated was 95 per cent in 24 hours and 100 per cent in 7 days. When the same degree of trauma was followed within 60 seconds by immersion of the burned area in cold water for 10 minutes, the mortality was reduced to 37 per cent in 24 hours and 88 per cent in 7 days. Figure 1 illustrates these results.

Further study revealed that cooling in 10° C water for 10 minutes reduced the intraperitoneal temperature of these burned mice from 100° F to 101° F to 68° F to 72° F and took on the average of 1½ hours for the body temperature to return to normal when the mice were placed in a warm atmospheric environment. Thus in these mice studies we have introduced a factor of generalized hypothermia in addition to the effects of local cooling. We have not as yet been able to determine whether the associated hypothermia was or was not a beneficial influence. We are now in the process

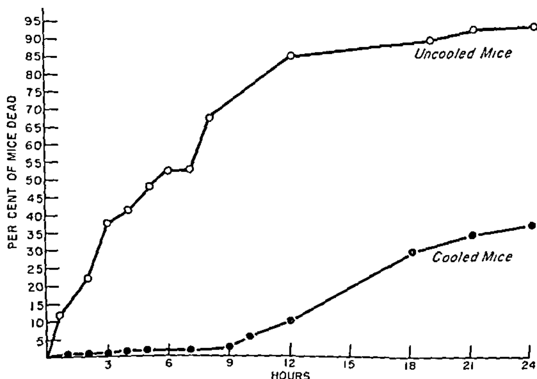


Fig. 1. The effect of cooling on the 24 hour mortality rate of the severely burned mouse.

Table 1

RATIO OF DILUTION	DEXTRAN : PLASMA	DEXTRAN : SERUM	DEXTRAN : FIBRINOGEN
1 : 1	ppt 0	0	+++
2 : 1	ppt +	0	+++
3 : 1	ppt ++	0	++++
4 : 1	ppt +++	0	++++

Table 2

DEXTRAN + BLOOD		NO. OF PLATELETS	PLASMA + BLOOD		NO. OF PLATELETS
1.0 cc	1.0 cc	++++	1.0 cc	1.0 cc	++++
1.35 cc	0.65 cc	++	1.35 cc	0.65 cc	++++
1.5 cc	0.5 cc	++	1.5 cc	0.5 cc	+++
1.6 cc	0.4 cc	+	1.6 cc	0.4 cc	+++

Table 3

DEXTRAN + BLOOD		CLOTTING TIME	SALINE + BLOOD		CLOTTING TIME
1.0 cc	1.0 cc	5 min	1.0 cc	1.0 cc	6 min
1.35 cc	0.65 cc	6 min	1.35 cc	0.65 cc	7 min
1.5 cc	0.5 cc	30 + min	1.5 cc	0.5 cc	8.5 min
1.6 cc	0.4 cc	30 + min	1.6 cc	0.4 cc	18 min
CONTROL 4 MINUTES			CONTROL 5 MINUTES		

substituted for plasma; conversely precipitation appeared when fibrinogen solution was diluted with dextran.

1b. Reduction in Number of Platelets. Varying amounts of dextran were added to citrated blood freshly drawn by the silicone technique to preserve platelets intact. After standing for 15 minutes a sample was taken from the center of each tube and a slide prepared for platelet estimation. Controls of plasma – whole blood mixtures were also set up. As shown in Table 2 the decrease in the number of platelets in the blood diluted with dextran is greater than in the controls. Reference to Table 1 indicates that the decrease in platelets is related to precipitation of fibrinogen.

1c. Prolongation of Clotting Time. Dextran and whole blood were mixed in the same proportions as in the previous experiment. The time necessary for the mixture to clot was noted. Volumes were kept constant and tests were performed at 37 C. As shown in Table 3 there is a progressive prolongation of the clotting time as increasing amounts of dextran are added to whole blood. The prolongation is greater than in controls of equivalent dilutions of saline and blood. The effect therefore is not due simply to dilution.

The Relationship of Abnormal Bleeding in Surgical Patients to Administration of Dextran, Excesses of Citrate and to Fibrinolysis

STUDIES ON THE BLEEDING TENDENCY FOLLOWING DEXTRAN INFUSION*

PAUL G WEIL AND DONALD R WEBSTER

Intravenous infusion of substances of large molecular weight may give rise to the macromolecular syndrome Among the hematologic aspects of the syndrome are a prolonged coagulation time thrombocytopenia and decrease in the fibrinogen content of the blood [2] There have been several recent reports of an increased bleeding tendency following the administration of dextran a macromolecular polysaccharide frequently used in place of blood as a plasma volume expander [1] The increased bleeding tendency may occur with infusions of dextran in amounts of 1500 cc or more

In a previous investigation of the use of dextran in the prevention and treatment of hemorrhagic shock in more than 500 cases no reactions or untoward effects of any kind were observed [4] Although no bleeding tendencies were noted they were not specifically sought for and may have been overlooked The present investigations were undertaken (1) to test *in vitro* the effects of various dilutions of dextran on (a) the fibrinogen level of plasma (b) the thrombocyte content of whole blood and (c) the clotting time of blood and (2) to determine by clinical and laboratory observations any increased bleeding tendency following the intravenous infusion of dextran in amounts of 500 to 2000 cc

EXPERIMENTAL

1a Precipitation of Fibrinogen Increasing amounts of dextran were added to a constant amount of fresh plasma The mixtures were allowed to stand at 37° C for 2 hours when they were examined for a precipitate Controls of serum and fibrinogen solution to which dextran was added were also set up As shown in Table 1 fibrinogen is precipitated from plasma in increasing amounts depending on the ratio of dextran to plasma As shown in the table there was no precipitation when equivalent amounts of serum were

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Table 1

RATIO OF DILUTION	DEXTRAN : PLASMA	DEXTRAN : SERUM	DEXTRAN : FIBRINOGEN
1 : 1	ppt 0	0	+++
2 : 1	ppt +	0	+++
3 : 1	ppt ++	0	++++
4 : 1	ppt +++	0	++++

Table 2

DEXTRAN + BLOOD		NO. OF PLATELETS	PLASMA + BLOOD		NO. OF PLATELETS
1.0 cc	1.0 cc	++++	1.0 cc	1.0 cc	++++
1.35 cc	0.65 cc	++	1.35 cc	0.65 cc	++++
1.5 cc	0.5 cc	++	1.5 cc	0.5 cc	+++
1.6 cc	0.4 cc	+	1.6 cc	0.4 cc	+++

Table 3

DEXTRAN + BLOOD		CLOTTING TIME	SALINE + BLOOD		CLOTTING TIME
1.0 cc	1.0 cc	5 min	1.0 cc	1.0 cc	6 min
1.35 cc	0.65 cc	6 min	1.35 cc	0.65 cc	7 min
1.5 cc	0.5 cc	30 + min	1.5 cc	0.5 cc	8.5 min
1.6 cc	0.4 cc	30 + min	1.6 cc	0.4 cc	18 min
CONTROL 4 MINUTES			CONTROL 5 MINUTES		

substituted for plasma; conversely, precipitation appeared when fibrinogen solution was diluted with dextran.

1b. **Reduction in Number of Platelets.** Varying amounts of dextran were added to citrated blood freshly drawn by the silicone technique to preserve platelets intact. After standing for 15 minutes a sample was taken from the center of each tube and a slide prepared for platelet estimation. Controls of plasma – whole blood mixtures were also set up. As shown in Table 2, the decrease in the number of platelets in the blood diluted with dextran is greater than in the controls. Reference to Table 1 indicates that the decrease in platelets is related to precipitation of fibrinogen.

1c. **Prolongation of Clotting Time.** Dextran and whole blood were mixed in the same proportions as in the previous experiment. The time necessary for the mixture to clot was noted. Volumes were kept constant and tests were performed at 37 C. As shown in Table 3, there is a progressive prolongation of the clotting time as increasing amounts of dextran are added to whole blood. The prolongation is greater than in controls of equivalent dilutions of saline and blood. The effect therefore is not due simply to dilution.

2 Observations were made in 2 groups of 50 patients who received dextran in amounts ranging from 500 to 2000 cc for blood loss at operation One group received dextran alone in amounts of 500 to 1000 cc In the other group which was given dextran in amounts of 1500 to 2000 cc whole blood was also administered Using a capillary bleeding time technique blood samples were obtained before 1, 3 and 24 hours after infusion of dextran The bleeding time in all cases was found to vary only within the normal limits of 3 5 to 5 5 minutes

DISCUSSION

Dextran has been shown *in vitro* to interfere with the normal mechanism of blood coagulation by precipitation of fibrinogen reduction in platelets and prolongation of the clotting time It has recently been reported that dextran *in vitro* retards the formation of thrombin in the clotting process[3] *It is possible therefore that dextran produces a bleeding tendency through* its effect on several or all of the factors involved in coagulation

No pathological bleeding or increased bleeding tendency was observed or could be detected in patients following infusion of dextran for the treatment or prevention of shock The absence of such an effect even in cases where as much as 2000 cc of dextran were used may be attributed to the concomitant use of whole blood since the dextran used was similar in quality and quantity to that reported by others to be the cause of a hemorrhagic tendency

It is emphasized furthermore that in the patients studied dextran was administered for the prevention or treatment of shock due to hemorrhage occurring normally at operation Because of its *in vitro* effects on coagulation no patient with pathological bleeding due to coagulation defects e g thrombocytopenia hypoprothrombinemia were included in this study

CONCLUSION

Dextran was found *in vitro* to precipitate fibrinogen from plasma and to decrease the number of platelets and to prolong the coagulation time in blood No tendency toward pathological bleeding or increase of bleeding *time could be found in 2 groups of 50 patients who received dextran for* the prevention or treatment of shock due to hemorrhage at operation One group received only dextran in amounts of 500 to 1000 cc The other group was given dextran in amounts of 1500 to 2000 cc supplemented with whole blood Providing certain precautions are observed i e the supplemental use of whole blood with dextran is administered in amounts over 1 liter and its contraindication in any amount in diseases with coagulation defects dextran is a reliable and safe plasma volume expander in the treatment of shock

REFERENCES

1 Adelson E Crosby W H and Roeder W H J Laborat Clin M *45* 441 1955
2 Hueper W C Arch Path Chic *33* 267 1942
3 Seegers W G Levine W G and Johnson S A J App Physiol *7* 617 1955
4 Weil P G and Webster D R in Surgical Forum 1952 Philadelphia W B Saunders Co 1953 p 712

BLEEDING AFTER LARGE TRANSFUSIONS OF CITRATED BLOOD*

An Experimental Study

WILLIAM B. KIESEWETTER AND JACKSON HARRIS

The free use of blood in the surgical procedures of today is an accepted practice. Only when an aberration of the hemostatic mechanism occurs do we stop and question its use and possible relationship to the hemorrhagic phenomenon. This study was undertaken because of encountering such a bleeding diathesis in 3 patients who received massive transfusions in the course of surgery.

Six factors seem apparent as possible causes in the bleeding following large-scale transfusions of citrated blood: (1) the action of the citrate solution on platelets to prevent their primary action in the coagulation mechanism; (2) the speed of the transfusion; (3) the temperature of the blood; (4) the age of the blood; (5) shock as a factor in the bleeding; (6) the action of the citrate solution in reducing the calcium ions below that level necessary for coagulation.

An experimental situation was set up in mongrel dogs to try to reproduce the bleeding tendencies seen in patients. Twenty-six dogs, weighing from 1.9 kg. to 21 kg., were employed, and a surgical wound was made in the femoral area of each dog so as to cannulate the femoral artery and vein for exsanguination, infusion, transfusion, and determination of blood pressure. The citrate solution used was identical with that used in the Central Blood Bank — 75 cc. per 175 cc. of blood with a concentration of 0.78 per cent citric acid, 2.3 per cent dextrose, and 2.1 per cent sodium citrate. The blood used was collected from reservoir dogs and refrigerated in this citrate solution. Blood was designated as "new" if it was drawn less than 2 days before use, that which was drawn 3 to 7 days before use being termed as "old." No blood over 7 days of age was utilized. The speed of transfusion was maintained either by syringe pumping or by regulation of the drops per minute which the animal received. The temperature of the blood used was controlled by instantaneous use or by allowing it to stand at room temperature for a period of time.

Preoperative and postoperative studies were made of bleeding time, clotting time, platelet count, clot retraction, prothrombin time, and bleeding tendency. Bleeding time was measured from a prick in the dog's ear; clotting time was measured by the capillary method of Kruse[1]; direct chamber platelet counts were done with Rees-Ecker fluid; clot retraction was observed in a capillary tube; prothrombin time was measured by the method of Quick; bleeding tendency was determined by observation for oozing from the operative wound after all bleeders had been tied and a dry field obtained. In the first 20 dogs all of the above studies were done. The last 6 dogs simply had platelet counts and an indirect attempt at an ionizable calcium determination by the method of McLean and Hastings, utilizing the relationship between total calcium and total serum protein.

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Our first effort was to produce the bleeding tendency if possible by giving the citrate solution to unanesthetized dogs so that we could see if tetany which might be masked by anesthesia preceded the bleeding. Amounts were used equivalent to the amount of citrate that would be present in 100 per cent replacement of blood volume by citrated blood. This was calculated from the dog's weight by estimating the dog's blood volume to be 10 per cent of his body weight. All 5 dogs oozed in manner similar to patients receiving massive transfusions. We found that bleeding occurred in the 3 cases in which no general anesthetic was used without any manifestation of tetany. Since the procedure without anesthesia was so much more difficult than with it, we felt justified in continuing our experiments under anesthesia.

We next used each of the various constituents of the citrate solution individually to see their effect upon producing the same bleeding diathesis. Only the 2.4 per cent sodium citrate reproduced the bleeding seen in patients. 4 of 6 such dogs bled.

We then attempted to reproduce the bleeding by exsanguination and transfusion of citrated blood. We chose 50 per cent, 60 per cent and 100 per cent replacement by transfusion, again calculating the amount necessary

Table 1

AGENT	OOZING	WARM	COLD	OLD	NEW	FAST	SLOW	SHOCK
Citrate Solution	Yes	+					+	
100% Replacement	Yes	+					+	
	Yes	+				+		
	Yes	+				+		
	Yes	+					+	
0.78% Citric Acid	No	+				+		
100% Replacement	No	+					+	
	No		+				+	
	No	+				+		
2.3% dextrose	No	+					+	
100% Replacement	No	+					+	
2.4% Sodium Citrate	Yes	+					+	
100% Replacement	Yes	+				+		
	No	+				+		
	Yes	+					+	
	No		+				+	
	Yes		+				+	
Citrated Blood	Yes	+		+			+	+
50% Replacement	No	+			+	+		+
60% Replacement	Yes	+		+			+	+
	Yes		+		+	+		+
	No		+	+			+	+
100% Replacement	Yes	+			+		+	+
	No	+			+		+	+
Fresh Non Citrated Blood	No	+			+		+	+
60% Replacement	No	+			+	+		+

Table 2

— = normal pre- and postoperative
↑ = elevated postoperative
↓ = decreased postoperative

AGENT	OOZING	BLEEDING TIME	CLOTTING TIME	CLOT RETRACTION	PROTHROMBIN TIME	PLATELET COUNT	UNSTABLE FACTOR
Citrate Solution 100% Replacement	Yes	—	—	—	—	↓	
	Yes	—	—	—	—	↓	
	Yes	—	—	—	—	↓	
	Yes	—	—	—		↓	
	Yes	—	—	—	—	↓	
0.78% Citric Acid 100% Replacement	No	—	—	—	—	↓	
	No	—	—	—	—	↓	
	No					↓	↓
	No					↓	↓
25% Dextrose 100% Replacement	No	—	—	—	—	↓	
	No	—	—	—	—	↓	
2.4% Sod. Citrate 100% Replacement	Yes	—	—	—	—	↓	
	Yes	—	—	—	—	↓	
	No					↑	↑
	Yes					↓	↑
	No					↓	↑
	Yes					↑	↑
Citrated Blood 50% Replacement	Yes	—	—	—	↑	↑	
	No	—	—	—	—	↓	
60% Replacement	Yes	—	—	—	↓	—	
	Yes	—	—	—	↓	—	
	No	—	—	—	↓	—	
100% Replacement	Yes	—	—	—	↓	↑	
	No	—	—	—	↓	↑	
Fresh non-citrated blood 60% Replacement	No	—	—	—	↑	—	
	No	—	—	—	↓	—	

by the dog's weight. It was found relatively impossible to exsanguinate the dogs initially to greater than 60 per cent of their estimated blood volume so that those dogs in whom a greater percentage of blood than 60 per cent was removed received transfusions of citrated blood during the withdrawal of any additional per cent of blood volume. We found that 1 of 7 such exsanguinated dogs definitely showed a bleeding tendency.

Finally, as a control, 2 dogs were 60 per cent exsanguinated and transfused with fresh unpreserved blood to be sure that simple transfusion did not cause oozing. Neither of these dogs bled.

Table 1 summarizes the results obtained in each of the categories of dogs done. Briefly, it shows no direct relationship between speed of infusion, the age of the blood, or the temperature of the blood and the bleeding tendency. Likewise, though all exsanguinated dogs showed shock-like levels of blood pressure, it bore no constant relationship to the bleeding picture since 1 of 7 shocked dogs bled and 3 did not.

Table 2 shows no significant and constant alteration in bleeding time clotting time or clot retraction The prothrombin time was altered in only 4 instances, 1 of which was an obviously sick dog whose prothrombin time rose to 21 seconds In 3 other instances the prothrombin time was altered — twice it was prolonged 1 second and once 3 seconds — 2 of these dogs showing a bleeding tendency and 1 failing to do so Platelet counts uniformly showed a decrease but they were never below 76 000, regardless of the procedure Six dogs had total calcium and total protein determinations done Four showed an increase of total calcium and all showed a lowering of total protein The significant finding was that the 2 dogs who showed the bleeding tendency showed no increase or an actual decrease in total calcium

It appears then that the citrate solution and more specifically, the sodium citrate in that solution, was the causative factor in the bleeding seen in this experimental situation Any discussion of the hemorrhagic tendency would seem to hinge on a discussion of the mechanism of action of the citrate solution *In vitro,* clotting is prevented by the binding of free calcium ions by the citrate or by establishing a calcium citrate ratio incompatible with coagulation Sodium citrate and citric acid both form complex salts to bind calcium[3] and on this basis it was expected that when the individual constituents of the citrate solution were investigated, both sodium citrate and citric acid would cause the bleeding tendency The fact that sodium citrate did cause the bleeding and citric acid did not cannot be readily explained The thought that free Ca was reduced to such an extent that coagulation would not occur is an intriguing one, and yet in the 3 instances in unanesthetized dogs in which citrate solution was given and bleeding occurred there was no tetany The amount of calcium necessary for coagulation is thought to be well below the critical level for tetany Furthermore the 6 dogs who had total calcium and total protein determinations run showed interesting results All dogs showed a decreased total protein The 1 dogs who did not bleed all showed marked increases in total calcium but the 2 dogs who bled showed no increase or a loss in total calcium These observations suggest that protein bound calcium is decreased but is compensated for by the increase of total calcium so that the amount available for ionization is increased in all cases However, the 2 dogs who bled showed no compensatory increase in total calcium and therefore showed the least availability of Ca at the time of the bleeding It is interesting to postulate that a given amount of sodium citrate was binding equal amounts of Ca in bleeding and non bleeding dogs This reduced the Ca , in the case of the bleeding dogs below a critical level for hemorrhage but did not do so in non bleeding dogs who started at a higher level of available Ca Direct ionizable calcium determinations are needed to confirm this postulation and such are planned in future work

Since all the quantitative tests for determining alterations of blood coagulability were normal in this experiment, another mechanism than altered coagulability may be operative Intercellular capillary cement is a material consisting of a reversible calcium salt[4] perhaps the citrate binds the calcium of this cement substance to allow capillaries to pass red cells

Aside from the role of calcium there is the question of the destruction of platelets This thought has been introduced by others who have observed

this bleeding, and two recent reports[5,6] indicate that there is a direct relationship between massive transfusions, the resultant thrombocytopenia and bleeding. Others have found that the injection of citrate will destroy platelets[7] or at least alter them in such fashion as to change their action in coagulation. Some have suggested that perhaps the substance in normal platelets which causes vasoconstriction at the bleeding site cannot be elaborated by citrated platelets. We cannot answer this thrombocytic concept of the etiology of the bleeding, except to say that while 8 of 13 bleeding dogs showed a platelet depression, in none of our animals were there sufficiently lowered platelets to account for the bleeding.

SUMMARY

The problem of a bleeding tendency following massive transfusions of citrated blood has been presented and an experimental situation in dogs has been reviewed to determine possible causes.

The speed of the transfusion, the temperature and the age of the blood and shock levels of blood pressure are not thought to be etiologic factors, whereas the citrate solution used to preserve the blood has been incriminated.

The mechanism of this citrate bleeding relationship is as yet obscure, for bleeding times, clotting times, clot retraction times, prothrombin times, platelet counts and ionizable calcium levels are not consistently abnormal in this experimental situation.

Further studies are in progress to try to elaborate the problem.

REFERENCES

1. Kruse, T. K., and Moses, C.: A method for determining coagulation, using capillary blood. J. Laborat. Clin. M., *30*: 631, 1945.
2. McLean, F. C., and Hastings, A. B.: The conditions affecting the ionization of calcium. J. Biol. Chem., *180*: 285, 1935.
3. Nicolaysen, R., and Norb, R.: Calcium metabolism and citric acid. Acta physiol. scand., *5*: 212, 1942-43.
4. Zweifach, B. W.: The structural basis of permeability and other functions of blood capillaries. Sympos. Quant. Biol., *8*: 216, 1940.
5. Stefanini, M., Medinicoff, I. B., Salomon, L., and Campbell, E. W.: Thrombocytopenia of replacement transfusion. A cause of surgical bleeding. Clin. Res. Proc., *2*: 61, 1954.
6. Krevans, J. R., and Jackson, D. P.: Hemorrhagic disorder following massive whole blood transfusions. J. Am. M. Ass., *159*: 171, 1955.
7. Neuhof, H., and Hirshfeld, S.: The intramuscular injection of sodium citrate. A new method for the control of bleeding. Ann. Surg., *76*: 1, 1922.

CAUSE AND PREVENTION OF HEMORRHAGE FOLLOWING EXTRACORPOREAL CIRCULATION*

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Great strides have been made in the past 2 or 3 years in the clinical application of several kinds of extracorporeal circulation for intracardiac surgery.[1] Yet when the blood is taken out of the body and especially if it is mechanically oxygenated, the blood's coagulation apparatus often appears to be damaged, so that severe hemorrhage may follow the perfusion. Such hemorrhage has been blamed on a great number of factors, from heparin to bacterial contamination, but in our experience it appears to be more directly related to direct damage to the blood in the mechanical circuit, such as by excessive turbulence, mechanical crushing, jets, eddies forming or exposure to improperly cleaned foreign surfaces.

METHOD

The data of this paper are derived from extracorporeal bypass of the heart and lungs in 90 dogs. Most of these perfusions were total heart-lung bypasses lasting from 20 to 50 minutes, often with a ventriculotomy. Heparin was usually given in a dose of 2.5 mg/kg and neutralized by protamine in a similar dose after the perfusion.

RESULTS

Our first 38 experiments were carried out using a mechanical pump-oxygenator consisting of 2 roller pumps and a rotating centrifugal oxy-

Table 1. 20 minute perfusion with roller pump rotating cone oxygenator. Mean values for 6 perfusions

% FALL IN PLASMA FIBRINOGEN	MG% HEMOLYSIS
41%	31

Table 2. 7 kg dog perfused at 500 cc/min without heparin

MIN. OF PERFUSION	CLOTTING TIME, MIN. (SILICONED TUBES)
Before	51
12	13
24	10
41	No clot

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genator. Although the perfusions appeared to be technically excellent with flows well over 50 cc/kg/minute of well oxygenated blood, almost all the animals died within 12 hours following the perfusion in shock with evidence of severe bleeding.

A search for the coagulation difficulty showed that in most cases plasma fibrinogen fell rapidly during the perfusion (see Table 1). To study this effect further we then perfused a number of dogs without using heparin. Fibrinogen levels fell in these dogs as in the others, but in this series we were able to follow the clotting time. Samples were taken in silicone coated tubes and it was found that clotting time at first decreased rapidly during perfusion (1 example is shown in Table 2) but then the blood became incoagulable. This is interpreted as a rough measure of increased activation of the coagulation process early in the perfusion followed by exhaustion of essential clotting elements. Another measure of activation of the coagulation process is the recalcification time of decalcified plasma. This also decreased markedly early in the perfusion.

In approximately half the animals perfused for more than 20 minutes plasma fibrinolytic activity developed. Fibrinolysins developed whether the animal had been heparinized or not. These changes in the clotting mechanism occurred even though our circuit as measured by the amount of hemolysis it caused was not particularly traumatic. Hemolysis after perfusion ranged usually from 25 to 40 mg per cent.

It seemed important at this time to determine whether trauma to the blood by excessively turbulent pumping alone without mechanical oxygenation could cause similar coagulation difficulty. A Sigmamotor pump[3] was obtained and with it blood was withdrawn from a dog's femoral vein and pumped directly back to the animal into the other femoral vein through a small cannula. The cannula size was so chosen that a flow rate of 400 cc/minute would require a pump pressure of 250 mm Hg to force the blood through the cannula. Perfusion was carried out for 90 minutes and blood samples were taken at intervals.

Table 3. Vein to vein perfusion by Sigmamotor pump. 7 kg dog. Flow 400 cc/min through small cannula, pressure 250 mm Hg

MIN OF PERFUSION	HEMOLYSIS MG%	WT OF CLOT GM
before	—	0.27
15	10	0.25
30	16	0.29
45	27	0.15
60	41	0.10
90	72	0.10

In Table 3 we present data from such an experiment. Note that at the end of the perfusion although plasma hemoglobin has risen to only 72 mg per cent, defibrination (as measured in this case by the weight of the clot) is very severe. We conclude from this type of experiment that pumping

blood at high pressure through small orifices with this type of pump can by itself cause defibrination similar to that caused previously by our complete pump-oxygenator circuit This defibrination following turbulent pumping seems to occur only *in vivo* that is if the animal is in the circuit A similar high pressure pumping circuit did not cause defibrination of citrated blood recirculated for a long period from bottle to bottle

Slower perfusions with the Sigmamotor pump through larger cannulas for shorter times were then explored To eliminate possible trauma from suction arterial blood was allowed to run out of a large catheter into a vessel from which it was returned to a different artery at the same rate through another large catheter by means of a Sigmamotor pump This type of circuit should be relatively non traumatic yet damage began to appear after about 30 minutes of pumping at flows near 400 cc./minute in 10 to 15 kg dogs Table 4 summarizes the findings from 5 such perfusions White cell counts and platelet counts began to fall Hemolysis ranged from 20 to 35 mg per cent Fibrinogen fell moderately

Table 4 Artery to artery perfusion with Sigmamotor pump Mean values for 5 consecutive dogs

	RED BLOOD COUNT	WHITE CELL COUNT	PLATELETS	(3 ANIMALS) (MG%) FIBRINOGEN
Before Perfusion	5.8 M	15,900	240,000	770
After 30-45 min Perfusion flow 300-400 cc./min	5.5 (Hemolysis 34mg%)	11,900	195,000	700
1-6 days later (4 dogs only)	3.6	28,500	240,000	

A further and unexpected finding was that although there was no evident change in the red blood count at the time of perfusion all animals suffered a severe anemia beginning the day after the perfusion Tagged red cell studies with radioactive chromium indicate that the loss in red cells in the day or days following the perfusion represented a loss both of the donor cells (used to prime the pump circuit) and of the operated animal's cells though the proportion of donor cells lost was higher It is as if the perfusion had destroyed outright only a few red cells (leading to the hemolysis found just after the perfusion) but had severely damaged a great many others which soon after the perfusion disintegrated and were lost

From data such as the above we concluded that trauma in the form of turbulence jets eddies etc should be avoided at all costs Furthermore at this time Ambrus and co-workers[4] showed a relation between foaming (or in fact any discontinuous blood film) and loss of white cells and platelets We have been able at least to partially confirm their results for the addition of a small amount of oxygen bubble foam to our Sigmamotor pump circuit appeared to increase the damage greatly In Table 5 are data from 2 perfusions which were slower than the perfusions of Table 4 but in which a small amount of foaming was introduced by oxygen bubbles followed by

Table 5. Before and after perfusion with Sigmamotor pump plus small amount of oxygen bubbles and defoaming (mean values for 2 perfusions)

	R.B.C. (MILLIONS)	W.B.C. (THOUSANDS)	PLATELETS
Before Perfusion	4.8	12.4	220,000
After 4[illegible] min perfusion at 250 cc/min	1	[illegible]	172,000

defoaming over silicone-coated plastic tubing. Fall in white count and platelet count was more severe in spite of the reduced rate of perfusion.

On the basis of information such as the above we determined to try to construct a pump-oxygenator in which, as far as possible, we would not tolerate turbulence, jets or foaming. Using hemolysis as a measure of trauma, we would aim for complete elimination of hemolysis. On this basis we constructed, after many tries, an air-driven pump-oxygenator circuit in which most surfaces are of silicone-coated glass, and which maintains a continuous film without foam or bubbles.

There is time here only to describe some of our preliminary results to indicate that our aims are not out of reach. We have carried out 18 complete heart-lung bypasses with our new apparatus and its forebears, from 20 to 30 minutes each (total perfusion time being usually 10 to 15 minutes). Hemolysis has often been under 5 per cent or unmeasurable within the experimental error of our technique. Fibrinogen has never been found to fall, and more recently there has been no fall in white count, though we still find a moderate drop in platelets. Fibrinolysins have not appeared. Bleeding has followed only 2 of these perfusions, and in both cases was associated with a serious mistake in perfusion technique.

CONCLUSIONS

1. We conclude then that bleeding following extracorporeal circulation is directly related to damage to the blood in the extracorporeal circuit, and is associated at first with increased activation of the coagulation apparatus and in its last stages with defibrination of the blood.

2. Excessive turbulence alone can cause severe damage. Bubbling and foaming appear to add to the damage.

3. Minimum damage is associated with moderate fall in white count and platelet count without other evidence of blood changes. With more severe damage, fibrinolytic activity appears. These changes do not all occur *in vitro*.

4. A pump-oxygenator perfusion circuit has been operated at extremely low levels of hemolysis, with no change in white count (though reduction in platelets has still occurred), and bleeding does not follow such perfusion.

REFERENCES

1. Kirklin, J. W., DuShane, J. W., Patrick, R. T., Donald, D. E., Hetzel, P. S., Harshbarger, H. G., and Wood, E. H.: Intracardiac surgery with the aid of a mechanical pump oxygenator system (Gibbon type). Report of 8 cases. *Proc. Mayo Clin.*, 30: 201, 19[illegible].
2. Newman, M. M., Stuckey, J. H., Levowitz, B. S., Young, L. A., Dennis, C., Fries, C., Gorayeb, E. J., Zuhdi, M., Karlson, K. E., Adler, S., and Chedman, M.: Complete and partial perfusion of animal and human subjects with the pump-oxygenator. *Surgery*, 38: 30, 19[illegible].

3 Cohen M Warden H E and Lillehei C W Physiologic and metabolic changes during autogenous lobe oxygenation with total cardiac by pass employing the azygos flow principle Surg Gyn Obst *98* 523 1954
4 Ambrus J L Ambrus C M Johnson G C and Harrison J E A simple heart lung apparatus not injurious to white cells and thrombocytes Presented to The American Society for Artificial Internal Organs Atlantic City 1955 (To be published)

STUDIES ON SERUM PROTEOLYTIC ACTIVITY IN PATIENTS WITH SPONTANEOUS FIBRINOLYSIS*

SAMUEL R POWERS JR AND HAROLD H BROWN

The activation of plasminogen to plasmin associated with fibrinolysis may lead to uncontrollable hemorrhage at the time of surgery This report will describe 3 factors which play a role in plasminogen activation

Such activation may occur either as a result of activation of plasminogen to plasmin or from the destruction of circulating antiplasmin Clinically significant fibrinolysis is a relatively rare phenomenon usually associated with major surgery and prolonged anesthesia Attention was directed to the effect of ether anesthesia and transfusions with blood containing hemolyzed red cells since these factors are frequently associated with major surgical procedures

The appearance of proteolytic activity after incubation of serum with chloroform or ether is well known [1] and is most probably due to inactivation of circulating anti plasmin In addition Macfarlane[2] has shown that lung tissue contains a plasminogen activator It therefore seemed possible that ether vapor in contact with the pulmonary parenchyma might induce fibrinolysis by depressing anti plasmin levels in an organ with considerable plasminogen activating potential

Because of uncertainty in the analysis of plasmin levels by fibrinolytic methods[3] the proteolytic activity of blood samples was assayed by casein digestion This method is less sensitive than clot digestion and the presence of proteolytic activity in the present studies is therefore considered to be significant [4] Plasminogen was determined by adding a crude extract of staphylokinase to dog blood and streptokinase to human blood It has been shown that the activation of plasminogen by these materials is stoichiometric and not enzymatic [5] and therefore an excess of activator must be present to make certain that complete activation has occurred The level of activity obtained in this way will depend not only on the amount of plasminogen but also on the level of available anti plasmin *In vitro* studies were carried out by incubating both plasma and serum with ether for varying periods of time In other studies the filtrate from washed dog red cells lysed with distilled water was added to plasma In a third set of experiments the 2 procedures were combined

In vivo studies were carried out by comparing plasmin and plasminogen

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concentrations in pulmonary arterial and pulmonary venous blood after varying periods of ether anesthesia.

RESULTS

The results of the *in vitro* studies are shown in Table I. After the addition of ether, increased proteolysis resulting from the addition of staphylokinase occurred, indicating a fall in the anti-plasmin level. After 1 hour incubation with ether alone, active plasmin was found to be present. This latter phenomenon was not observed in the etherized dogs which were studied.

The addition of hemolyzed red cells alone did not induce proteolytic activity. If, however, the anti-plasmin level was depressed by the addition of ether prior to the addition of lysed red cells, proteolysis invariably took place.

In vitro studies carried out on recalcified plasma when ether or hemolyzed red cells were added invariably showed greater proteolytic activity than identical experiments in which the reagents were added to untreated plasma. The reason for this is not clear and might be due to either the excess calcium ion present or to the decrease in fibrinogen as it is converted to fibrin. Initial experiments indicate that both factors may be important.

Table I. Effect of Ether and Hemolyzed Red Cells on Proteolytic Activity

I	Plasma Buffer	Plasma STRP	Hemolyzed red cells Buffer	Hemolyzed red cells STRP
Optical Density	0.015	0.520	0.020	0.020
II	Plasma Buffer Ether	Plasma STRP Ether	Hemolyzed red cells Buffer Ether	Hemolyzed red cells STRP Ether
Optical Density	0.130	0.975	0.010	0.010
III	Plasma Hemolyzed red cells		Plasma Hemolyzed red cells Ether	
Optical Density	0.015		0.390	
IV	Plasma Buffer	Plasma Buffer Ether	Recalcified Plasma Buffer	Recalcified Plasma Buffer Ether
Optical Density	0.005	0.020	0.005	0.160

3 Cohen M Warden H E and Lillehei C W Physiologic and metabolic changes during autogenous lobe oxygenation with total cardiac bypass employing the azygos flow principle Surg Gyn Obst *98* 523 1954
4 Ambrus J L Ambrus C M Johnson C C and Harrison J F A simple heart lung apparatus not injurious to white cells and thrombocytes Presented to The American Society for Artificial Internal Organs Atlantic City 1955 (To be published)

STUDIES ON SERUM PROTEOLYTIC ACTIVITY IN PATIENTS WITH SPONTANEOUS FIBRINOLYSIS*

SAMUEL R POWERS JR AND HAROLD H BROWN

The activation of plasminogen to plasmin associated with fibrinolysis may lead to uncontrollable hemorrhage at the time of surgery This report will describe 3 factors which play a role in plasminogen activation

Such activation may occur either as a result of activation of plasminogen to plasmin or from the destruction of circulating antiplasmin Clinically significant fibrinolysis is a relatively rare phenomenon usually associated with major surgery and prolonged anesthesia Attention was directed to the effect of ether anesthesia and transfusions with blood containing hemolyzed red cells since these factors are frequently associated with major surgical procedures

The appearance of proteolytic activity after incubation of serum with chloroform or ether is well known [1] and is most probably due to inactivation of circulating anti plasmin In addition Macfarlane[2] has shown that lung tissue contains a plasminogen activator It therefore seemed possible that ether vapor in contact with the pulmonary parenchyma might induce fibrinolysis by depressing anti plasmin levels in an organ with considerable plasminogen activating potential

Because of uncertainty in the analysis of plasmin levels by fibrinolytic methods[3] the proteolytic activity of blood samples was assayed by casein digestion This method is less sensitive than clot digestion and the presence of proteolytic activity in the present studies is therefore considered to be significant [4] Plasminogen was determined by adding a crude extract of staphylokinase to dog blood and streptokinase to human blood It has been shown that the activation of plasminogen by these materials is stoichiometric and not enzymatic [5] and therefore an excess of activator must be present to make certain that complete activation has occurred The level of activity obtained in this way will depend not only on the amount of plasminogen but also on the level of available anti plasmin *In vitro* studies were carried out by incubating both plasma and serum with ether for varying periods of time In other studies the filtrate from washed dog red cells lysed with distilled water was added to plasma In a third set of experiments the 2 procedures were combined

In vivo studies were carried out by comparing plasmin and plasminogen

* From the Departments of Surgery and Biochemistry Albany Medical College Albany New York

connected to a respirator Cooling and rewarming was carried out with a Thermo-Rite machine It was our intention to simulate as closely as possible the methods of cooling and rewarming which we were using on humans Temperatures were reduced to 18 to 25 C Dogs were kept at this temperature from 1 to 4 hours Rectal temperatures were taken with a mercury thermometer

For most studies arterial blood was used obtained from a polyethelene catheter in the femoral artery Some specimens were obtained from the femoral vein Some from both femoral vein and artery for comparison

Determinations were made of

1 Coagulation time using the method of Lee White
2 Bleeding time using a #11 Bard Parker blade in a cork on the under surface of the mid portion of the tongue
3 Hemoglobin using a Klett Summerson photoelectric colorimeter
4 Hemocrit using Wintrobe tubes
5 Platelet counts by the direct method using Rees Ecker solution and a red cell pipette
6 Clot retraction readings taken at 1 hour 4 and 24 hours
7 White blood cells
8 Prothrombin time in plasma one stage method using Simplastin
9 Prothrombin time in serum using the method of Stefanini and Crosby[17]
10 Osmotic fragility
11 Mechanical fragility using the method of Shen[14] with modifications by Greenwalt[8]
12 Critical fibrinogen index determination using Fibrindex
13 Heparin activity protamine heparin titration test after the method of LeRoy[10]
14 Smears of peripheral blood

Table 1 Coagulation time Bleeding time

	Coagulation time 34 dogs			Coagulation time 6 dogs						Bleeding time	
	M	SE	P	1	2	3	4	5	6	M	SE
Control				5 0	4 5	4 3	4.2	6 3	4.3		
36 38 C	6 6 min (34)	0 44	0 001							1 1 min (12)	6 4
31 34 C	6 8 min (24)	0 51	0 001							1 9 min (6)	2 8
water bath 24 25				6	3 0	5 0	5.5	3.5	3 3		
18 25 C	7.2 min (34)	0 47	0 001							5 8 min (12)	4 3
water bath 37 C				6 5	4 0	6 5	3 0	3.5	3.3		
31 34 C	6 8 min (21)	0 46	0 001							1 6 min (4)	2 4
35 37 C	5 7 min (32)	0 41	0 001	5	3 0	3 0	3 5	3 0		2 0 min (9)	3 5

( ) = Number of dogs tested M = Mean SE = Standard Error P = probability

SUMMARY

1 A plasminogen activator in low concentration has been found in hemolyzed human and dog red blood cells

2 This activation becomes manifest by incubating the serum with ether

3 It is suggested that ether anesthesia in conjunction with transfusions which produce significant hemolysis may lead to fibrinolysis

REFERENCES

1 Tagnon H J The significance of fibrinolysis in the mechanism of the coagulation of blood J Laborat Clin M 27 1119 1942
2 Macfarlane R G and Biggs R Fibrinolysis its mechanism and significance Blood Balt 3 1167 1948
3 Sherry S The fibrinolytic activity of streptokinase activated human plasmin J Clin Invest 33 1054 1954
4 Macfarlane R G and Pilling J Observations on fibrinolysis plasminogen plasmin and anti plasmin content of human blood Lancet Lond 2 567 1946
5 Wassermann A F Streptokinase activation of a proteolytic enzyme in human blood Arch Biochem N Y 41 158 1952

HEMATOLOGIC CHANGES IN HYPOTHERMIC DOGS*

CECIL MELVILLE COUVES ROBERT C OVERTON JR AND WAYNE L EATON

Hypothermia is being used widely in the field of Surgery [18 19 9 16 13 11 5 1 6] Various investigators have reported on the hematologic changes resulting from cooling Ross[1] Villalobos[9] Deterling[5] and Sircar[1] have reported increases in the clotting time as a result of cooling Delorme[4] speaks of the anti coagulant effect of hypothermia Most investigators have made these observations on dogs There is a sparsity of data on humans Dundee[7] reports 2 clinical cases of severe bleeding during the use of hypothermia In neither case did he feel that hypothermia had contributed to this in creased bleeding tendency Swan[18] reports on the use of hypothermia in 100 clinical cases He likewise does not incriminate hypothermia as a cause in those cases with postoperative hemorrhage

Prior to this study it was our observation that in some animals and in the occasional human although blood shed during surgery seemed to clot adequately there was an increased oozing tendency at cooled temperatures It was for the purpose of investigating this problem that we undertook our experimental studies

METHOD

Sixty seven mongrel dogs were used Most dogs received from 2 to 3 mg / kg of morphine sulphate intramuscularly before sodium pentobarbital (25 to 30 mg per kg) was given intravenously for anesthesia Additional small doses of morphine or sodium pentobarbital were given for shivering or for added anesthesia All dogs had an endotracheal tube passed and were

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connected to a respirator. Cooling and rewarming was carried out with a Thermo-Rite machine. It was our intention to simulate as closely as possible the methods of cooling and rewarming which we were using on humans. Temperatures were reduced to 18° to 25° C. Dogs were kept at this temperature from 1 to 4 hours. Rectal temperatures were taken with a mercury thermometer.

For most studies arterial blood was used, obtained from a polyethelene catheter in the femoral artery. Some specimens were obtained from the femoral vein. Some from both femoral vein and artery for comparison.

Determinations were made of:

1. Coagulation time, using the method of Lee White.
2. Bleeding time, using a #11 Bard Parker blade in a cork, on the under surface of the mid portion of the tongue.
3. Hemoglobin, using a Klett Summerson photoelectric colorimeter.
4. Hemocrit, using Wintrobe tubes.
5. Platelet counts by the direct method, using Rees Ecker solution and a red cell pipette.
6. Clot retraction, readings taken at 1 hour, 4 and 24 hours.
7. White blood cells.
8. Prothrombin time in plasma, one stage method using Simplastin.
9. Prothrombin time in serum, using the method of Stefanini and Crosby.[17]
10. Osmotic fragility.
11. Mechanical fragility, using the method of Shen[14] with modifications by Greenwalt.[6]
12. Critical fibrinogen index determination, using Fibrindex.
13. Heparin activity, protamine heparin titration test, after the method of LeRoy.[2]
14. Smears of peripheral blood.

Table I. Coagulation time. Bleeding time

	COAGULATION TIME 34 DOGS			COAGULATION TIME 6 DOGS	BLEEDING TIME	
	M	SE	P	1 2 3 4 5 6	M	SE
Control				5.0 4.5 4.5 4.2 6.3 4.3		
36–38° C	6.6 min (34)	0.44	0.001		1.1 min (12)	6.4
31–34° C	6.8 min (21)	0.71	0.001		1.9 min (6)	2.8
18–25° C	7.2 min (34)	0.47	0.001	water bath 24–25°: 6 3.0 5.0 5.5 3.5 3.3; water bath 37° C: 6.5 4.0 6.5 3.0 3.5 3.3	5.8 min (12)	1.3
31–34° C	6.8 min (21)	0.16	0.001		1.C min (4)	2.4
35–37° C	5.7 min (32)	0.44	0.001	r 3.0 3.0 3.5 3.0	2.0 min (9)	3.5

( ) = Number of dogs tested M = Mean SE = Standard Error P = probability

Table 2 Hemoglobin Hematocrit Platelets Clot retraction Leukocytes

	CONTROL 35 37 C		31 34 C	18 25 C	31 34 C		35 37 C
				HEMOGLOBIN			
M	(8)	10 4		(8)	11 0	(7)	14 0
S E and P	0 68	001		0 19	001	0 53	001
				HEMATOCRIT			
M	(12)	37 9		(12)	11 4	(6)	42 1
S E and P				2 6	001	1 9	001
				PLATELETS			
M	(11)	151 800		(11)	42 709	(10)	102 000
S E and P	10 601	001		12 869	001	21 898	001
				CLOT RETRACTION			
Dog 1	+++	1 hr	++1 hr		0 1 hr	++	1 hr
Dog 7	+++	1 hr	+1 hr		0 1 hr	++	1 hr
				LEUKOCYTES			
M	(10)	11 092		(12)	3 047	(7)	11 035
S E and P	1438	001		4 9	001	1654	001
( ) = Number of dogs		M = Mean		S E = Standard error		P = probability	

Table 3 Prothrombin time in plasma Prothrombin time in serum Osmotic fragility Mechanical fragility, critical fibrinogen index

	CONTROL 35 37 C		31 34 C		18 25 C		31 34 C		35 37 C	
					PROTHROMBIN TIME IN PLASMA					
M	(12)	8 5 sec			(12)	6 7 sec			(9)	8 2 sec
S E and P	28	001			15	001			35	001
					PROTHROMBIN TIME IN SERUM					
M	(6)	52 4 sec			(6)	18 1 sec			(5)	27 2
S E and P	6 7	01			(8)	001			2 7	001
					OSMOTIC FRAGILITY					
M	(10)	0 41			(11)	0 41			(9)	0 42
					MECHANICAL FRAGILITY					
M	(11)	54 1%			(12)	43 7%			(8)	32 8%
					CRITICAL FIBRINOGEN INDEX					
M	(13)	24 sec	(13)	25 sec	(14)	28 sec	(11)	23 sec	(10)	27 sec
S E and P	1 76	001	2 37	001	3 08	001	2 12	001	2 75	0 01
( ) = Number of dogs			M = Mean			S E = Standard error			P = probability	

Table 4 Heparin Activity

	CONTROL 35 37 C			18 25 C				35 137 C			
Dog 10	17 4	16 5	16 7	17 8	18 0	17 2	16 4	17 8	17 6	17 4	17 1
	16 2	15 8	15 5	16 7	16 4	16 1		16 9	16 4	16 4	
Dog 11	16 2	16 2	15 6	17 4	18 0	16 9	16 5	17 0	14 8	15 9	15 0
	15 3	15 3	14 8	16 3	16 7	15 9		15 2	15	14	14 5

RESULTS

Coagulation Time This was carried out in 10 dogs In the first 31 times were measured in water at 37.5 C In the last 6 times were measured simultaneously in two baths one at the reduced temperature of the dog and one at 37 C From Table 1 it is seen that there are no significant changes as a result of cooling Although not recorded in the table the rate of cooling length (10 of these dogs were kept at the maximum depth of cooling for 4 hours) or the length of rewarming altered these estimations The results as measured in a cooled water bath do not differ from those carried out in one at 37.5 C

Bleeding Time From Table 1 we observed that the average of the bleeding times at lowered temperatures was significantly increased over the control Of more importance was the fact that in 5 of the 12 dogs the bleeding time at cooled temperatures was more than 7 times the control value In one dog the bleeding time was 17 minutes The bleeding times returned to normal when the animal was rewarmed

Hemoglobin and Hematocrit These both increased with cooling This is in keeping with the work of Villalobos Strear[15] and Deterling[3]

Platelet and Clot Retraction The platelet count dropped with cooling Some animals had counts of 10 000 or less at 20 C With this drop the clot retraction was poor (Table 2)

White Blood Cells. There was a precipitous drop at lowered temperatures

Prothrombin Time—In Plasma and Serum The plasma prothrombin times were not altered by cooling however the serum prothrombin times are lowered at reduced temperatures probably due to low platelet counts at this temperature

Osmotic Fragility Mechanical Fragility, Critical Fibrinogen Index, Heparin Activity These were unchanged by cooling

Smears This was carried out on 5 animals There was at the depth of cooling a relative increase in the lymphocytes in 1 of the 5 dogs There were no other striking changes

DISCUSSION

Because the coagulation time and prothrombin estimation in plasma were both unaffected by cooling we do not feel that hypothermia seriously affects the over all coagulation mechanism This is in keeping with our clinical observation on cooled animals and humans that blood shed during surgery seems to clot adequately

We feel the prolonged bleeding times and reduced platelet counts at lowered temperatures are important Bleeding time is a measure of the capillary response to trauma We feel the increase in bleeding time may be due to (a) altered capillary response to trauma as a result of cold (b) increase in venous pressure (c) reduced platelet count

However it is also apparent that although the prolongation in the bleeding time at reduced temperature is consistent the number of animals in which it is prolonged to a dangerous degree is few This would support the clinical observations that very few animals show evidence of a marked bleeding tendency Furthermore even if this process is evident at lowered temperatures it is completely reversible when the animal is rewarmed

REFERENCES

1 Bailey C P Cookson B A Downing D F Neptune W B Cardiac surgery under hypothermia J Thorac Surg *27* 23 91 1954
2 Blades B Pierpont H C A simple method for inducing hypothermia Ann Surg *140* 557 1954
3 Cookson B A Neptune W Bailey C P Intracardiac surgery with hypothermia J Internat Coll Surgeons *18* 684 1952
4 Delorme E J Controlled hypothermia Postgrad M J Lond *31* 456 1955
5 Deterling R A Jr Nelson E Bhonslay S Howland W Study of basic physiologic changes associated with hypothermia A M A Arch Surg *70* 87 1955
6 Downing D F Cookson B A Keown K K Bailey C P Hypothermia in Cardiac Surgery J Pediat *44* 134 141 1954
7 Dundee J W Gray T C Mesham P R Scott W E B Hypothermia with autonomic block in man Brit M J *12* 1213 1953
8 Greenwalt T J Triantaphyllopoulos D C In vitro studies of red cell fragility in anti D hemolytic disease of the newborn J Laborat Clin M *45* 135 1955
9 Hardin C A Reismann K R Dimond F G The use of hypothermia in resection and homologous graft replacement of the thoracic aorta Ann Surg *140* 720 1954
10 LeRoy G V Halpern B Dolkart R F An indirect quantitative method for the estimation of heparin activity in vitro The heparin protamine titration test J Laborat Clin M *35* 446 1950
11 Lewis F J Taufic M Closure of atrial septal defects with the aid of hypothermia experimental accomplishments and the report of one successful case Surgery *33* 52 59 1953
12 Ross D N Hypothermia Guys Hosp Rep Lond *103* 116 1954
13 Scott H W Jr Collins H A Foster J H Hypothermia as an adjunct in cardio vascular surgery experimental and clinical observations Am Surgeon *20* 799 1954
14 Shen S C Castle W B Fleming F M Experimental and clinical observations on increased mechanical fragility of erythrocytes Science *100* 387 1944
15 Sircar I Plasma volume bleeding and clotting time on hypothermic dogs Proc Soc Exp Biol N Y *87* 194 195 1954
16 Smith A Fairer J G Hibernation anaesthesia in major surgery a report of 56 cases Brit M J *2* 1247 1953
17 Stefanini M Crosby W H Serum prothrombin time a composite effect an analysis of the factors involved Am J Clin Path *20* 1026 1950
18 Swan H Virtue R W Blount S G Jr Kircher L T Jr Hypothermia in surgery Ann Surg *142* 382-400 1955
19 Trusler G A McBirnie J F Pearson F G Gornall A G, and Bigelow W G A study of hibernation in relation to the technique of hypothermia for intra cardiac surgery in Surgical Forum 1953 Philadelphia W B Saunders Co 1954 pp 72 77
20 Villalobos T J Adelson E Barila T G Hematalogic changes in hypothermic dogs Proc Soc Exp Biol N Y 89 192 1955

HEPATIC HYPOTHERMIA AND ARTERIALIZATION IN ISCHEMIC SHOCK FOLLOWING TEMPORARY OCCLUSION OF THE THORACIC AORTA*

MAX BEN, WILLIAM M. PARKINS AND HARRY M. VARS

Surgical procedures necessitating total or partial arrest of the circulation has made it increasingly important to evaluate the factors which may increase the tolerance time of certain vital organs to deprivation or critical reduction of their blood supply. Maintenance of the integrity of the hepatic circulation has been frequently suggested as being one of the major factors in the prevention of shock and shock-like states. Perfusion of the liver during hemorrhagic shock has produced beneficial effects according to several investigators[1-4]. Others by cross-circulation and auto-perfusion of the liver of dogs subjected to hemorrhagic shock have been unable to prevent irreversible shock[5,6].

Edwards *et al*[7] in their experiments of cross-clamping the aorta with the hepatic circulation intact found only a minor beneficial effect in decreasing the mortality compared to that of cross-clamping the thoracic aorta above the celiac depriving the liver of its blood supply. Burch *et al* occluded the thoracic aorta above the celiac in the normothermic animal for 60 minutes with 100 per cent deaths whereas all 8 animals that had their liver cooled selectively survived. Generalized hypothermia has been reported to increase the time tolerance of the liver to temporary ligation of its afferent circulation[9].

Since the role of the liver in shock and shock-like states is equivocal, we were concerned whether the liver was or was not a major factor in the morbidity and mortality observed in ischemic shock induced by temporary occlusion of the thoracic aorta. If a reduction of the circulation and thus its oxygen supply to the liver is a major factor in this form of shock, would arterialization prove of value? Also, as an adjunct to arterialization, would reduction of the tissue demand for oxygen by hypothermia decrease the observed mortality?

METHOD

Adult dogs were anesthetized with pentobarbital sodium (25 mg./kg.) and subsequently heparinized. Blood sampling, supplementary nembutal injections or fluid administration was accomplished by means of a polyethylene catheter introduced via the right jugular vein into the superior vena cava. The left carotid artery was catheterized with a polyvinyl catheter attached to a triple arm U tube. The left jugular vein was catheterized with a 15 foot polyvinyl catheter, one end of which was attached to one arm of the U tube, thus establishing a carotid-jugular shunt. Carotid blood pressures were obtained with a mercury manometer connected to an arm of the U tube.

Both femoral arteries were isolated. A balloon catheter was introduced into the left femoral artery and the balloon positioned at the eighth to tenth

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intervertebral space The balloon catheter and placement of the catheter has been described previously[10] The right femoral artery was catheterized with a polyvinyl catheter (attached to a mercury manometer) the tip of which was just distal to the bifurcation of the descending abdominal aorta

Under aseptic conditions a catheter was introduced into the splenic vein and positioned in the portal vein at its entrance to the liver One end of a 30 foot polyvinyl catheter (capacity 70 ml H_2O) was attached to the remaining limb of the U tube and the other to the portal vein catheter Some animals were allowed to auto perfuse their own livers without the aid of a pump In others the carotid portal shunt was inserted in a Sigma motor pump to maintain the volume of blood entering the liver at a controlled rate (20 cc /kg /min)

Temperatures were recorded with thermocouples in the left hepatic lobe and the carotid artery In some of the animals a thermocouple was also placed in the lumen of the duodenum Rectal temperatures were obtained with a mercury thermometer inserted 10 cm into the rectum Oral temperatures when taken were obtained with a mercury thermometer

The carotid jugular shunt was submerged in warm water (45 C) when necessary (to avoid excess blood cooling and cardiac arrest) In the hypothermic series the carotid portal shunt was immersed in an ice bath to obtain and maintain desired liver temperatures during the occlusion Cooling was started simultaneously with thoracic aorta occlusion After release of the obstruction the animals were rewarmed by forced air heat directed under the animal

Artificial respiration using compressed air via an endotracheal tube was instituted when necessary

After release of the aortic occlusion the portal vein catheter was removed and the abdomen closed in the usual fashion

Various types of measurements were made over a 3 hour period after release of the occlusion Those animals living and in good condition for 72 hours were considered as survivors

RESULTS

In all animals the thoracic aorta was temporarily occluded for 1 hour The average femoral pressure during the occlusion was approximately 16 mm Hg Table I summarizes some of the observed data in these experiments

Hepatic Arterialization Normothermic Seven of 9 animals succumbed following release of a 1 hour occlusion of the aorta 6 within 24 hours and 1 within 32 hours The majority of the animals exhibited hypotension hemoconcentration and the passage of bloody diarrhea and mucus from the rectum following release of the occlusion With 4 dogs of this series the livers were perfused with the Sigma motor pump Of this group 1 animal survived This surviving dog had extensor rigidity of the hind quarters and priapism Hemoconcentration hypotension and the discharge of bloody diarrhea and mucus was not observed in this animal following release of the aortic occlusion The 1 animal in this series that died within approximately 32 hours had hemoconcentration and hypotension following release of the occlusion Also on the following day paraplegia was present

Five dogs were permitted to perfuse their own livers without the aid of the pump One animal survived in this series This dog passed some mucus

Table 1 Hepatic Hypothermia and Arterialization in Ischemic Shock Following 1 Hour of Temporary Occlusion of the Thoracic Aorta

EXPERIMENT	NO OF DOGS	AVERAGE TEMPERATURE C. AT END OF OCCLUSION					NUMBER LIVING HOURS AFTER OCCLUSION RELEASE			NUMBER PARAPLEGIC
		ORAL	CAROTID	DUODENUM	RECTAL	LIVER	24	48	72	
Normothermic	10				37		4	4	4	4
Arterialized	4*				37		2	1	1	2
Normothermic	5				37		1	1	1	1
Arterialized	4*	24	21	31	30	20	4	3	3	0
Hypothermic	4				30	20	3	-	-	0

Controlled perfusion by use of a pump in the carotid portal shunt
Auto perfused (carotid portal shunt)

and blood tinged feces following release of aortic obstruction Hind quarter paralysis priapism and extension of the tail developed within 24 hours

Hepatic Arterialization Hypothermia Eight dogs were utilized in this series with 5 survivals and 3 deaths In contrast to the normothermic control animals bloody diarrhea and discharge of mucus via the rectum were seen in only 1 animal (slight mucus discharge) In 4 animals the liver was perfused with the aid of the pump and in the remaining 4 liver perfusion was accomplished without mechanical assistance The temperature of the liver in both groups of animals was lowered to approximately 20 C in about 9 minutes (range of 5 to 15 minutes) and then maintained at this level until the end of the 1 hour aortic occlusion The average temperatures observed at the end of aortic occlusion were rectum 30 C carotid 21° C duodenum 31° C and oral 21° C respectively In the series which mechanical perfusion of the liver was used 3 animals survived and 1 died within 18 hours Three hours after release of the occlusion a moderate hypotension was noted The rectal temperatures at this time averaged 33° C The average hematocrit increased 13 percentage points Bloody diarrhea mucus discharge and paraplegia were not observed in any of these animals

In the auto perfused group 2 of 4 animals survived while 1 dog was found dead within 24 hours after release of the aortic obstruction and the other within 18 hours In this group the average liver temperature at the end of the occlusion was approximately 20° C and that of the rectum 30° C Hemoconcentration was moderate with the femoral pressure being most severely depressed in the animal dying within 18 hours A slight mucous discharge via the rectum was also observed in this animal No adverse neurological symptoms were seen in any of these animals

DISCUSSION

Increasing the blood flow thus oxygen supply to the liver did not improve the survival time over that observed in the control animals A decrease in severity of the ischemic shock was observed when both arterialization and hepatic hypothermia were employed together In the latter the degree of body core cooling was sufficient to prevent spinal cord damage The improved situation resulting after cooling of the liver could be due to an increased protection of this organ It also could be due in part to increased cooling of the intestine

A cardinal feature of dogs succumbing to this type of ischemic shock is the profuse bloody diarrhea and mucous discharge from the bowel In surviving dogs this either does not occur or is minimal It was minimal in the dogs treated with arterialization and hypothermia In view of these observations it appears that the role of the liver in the development of ischemic shock after aortic occlusion may be of lesser importance than the integrity of the extensive vascular bed of the intestine

Other experiments from this laboratory have shown that when the intestine is differentially cooled to 10 C to 20° C while the liver is reduced to 22 C to 28 C, all dogs survive 1 hour of occlusion[11] A large majority survived a 2 hour period of occlusion which was fatal in all normothermic controls[10, 11] These experiments as well as those reported by Edwards[7] suggest that the intestine rather than the liver may be the primary organ limiting the time tolerance of occlusion of the thoracic aorta

Summary

Hepatic arterialization (carotid-portal shunt) during 1 hour of thoracic aorta occlusion was studied in 9 animals, 4 of which were perfused with a Sigma motor pump. The tolerance to temporary occlusion of the thoracic aorta was not extended in this series as compared with a control series without shunts. Seven of the 9 animals in the experimental series succumbed of ischemic shock. Hemoconcentration, hypotension, bloody diarrhea and a mucus discharge was observed in the majority of these animals. Paralysis of the hind quarters was seen in the surviving normothermic animals.

Hepatic hypothermia with liver arterialization (blood refrigeration — liver 20 C, blood 21 C and rectum 30 C) with and without mechanical aid during obstruction of the thoracic aorta improved the morbidity and survival time of 8 animals. Five of 8 dogs survived following release of obstruction. Hemoconcentration and hypotension were not as severe as in their controls and bloody diarrhea with a mucus discharge was virtually absent. Paralysis of the hind quarters due to anoxial damage of the spinal cord was not observed in any of the hypothermic group.

References

1. Frank, H. A., Seligman, A. M., and Fine, J.: Traumatic shock XIII. The prevention of irreversibility in hemorrhagic shock by vivi-perfusion of the liver. J Clin Invest 25:22-29, 1946.
2. Hay, E. B., and Welch, J. K.: The effect of increased arterial blood flow to the liver in the mortality rate following hemorrhagic shock. Surgery 29:826-828, 1951.
3. Cohn, R., and Parsons, H.: Relationship of portal hypertension and irreversibility of shock. Am J Physiol., 160:437-440, 1950.
4. Delorme, E. J.: Arterial perfusion of the liver in shock. An experimental study. Lancet, London, 1:259-263, 1951.
5. Reinhard, J. J., Glasser, O., and Page, I. H.: Hemorrhagic hypotension in hepatectomized and bilaterally nephrectomized hepatectomized dogs. Am J Physiol., 155:106-115, 1948.
6. Wayne, H. H., Weir, A. F., Jr., Barnes, J. A., Joyner, J. T., III, Tuttle, D., Linde, T., and Green, H. D.: Interrelationship of liver perfusion, bacteremia and VDM in hemorrhagic hypotensive shock. Am J Physiol 176:301-310, 1954.
7. Edwards, W. S., Tidwell, O. K., and Lombardo, C. R.: The mechanism of death from thoracic aorta occlusion. In Surgical Forum 1954. Philadelphia, W. B. Saunders Co., 1955, pp. 90-95.
8. Burch, B. H., Traphagen, D. W., Folkman, M. J., Rosenbaum, D. A., and Mueller, F. C.: Temporary aortic occlusion in abdominal surgery. Surgery 35:684-689, 1954.
9. Raffucci, F. L., Lewis, F. J., and Wangensteen, O. H.: Hypothermia in experimental hepatic surgery. Proc Soc Exp Biol N Y 83:639-640, 1953.
10. Parkins, W. M., Ben, M., and Vars, H. M.: Tolerance of temporary occlusion of the thoracic aorta in normothermic and hypothermic dogs. Surgery 38:38-47, 1955.
11. Parkins, W. M., Ben, M., and Vars, H. M.: Visceral hypothermia in management of ischemic shock. Fed Proc Balt 14:111, 1955.

THE EFFECTS OF ACTH ON MEGAKARYOCYTE ACTIVITY IMPLICATIONS CONCERNING POSTOPERATIVE THROMBOPHILIA*

JOHN A WILLIAMS JOHN S BELKO AND RICHARD WARREN

The regular occurrence of an early thrombocytopenia and a late thrombocytosis following major operations[1] has led us to examine the hypothesis that these changes are normal features of the biologic response to surgery

The evidence that the early thrombocytopenia can be simulated in normal subjects by administration of ACTH[1] suggests that this phase of the platelet response to surgery may be determined primarily by adrenocortical hyperactivity

The present study was undertaken to evaluate this concept by comparing the altered patterns of megakaryocyte activity resulting from ACTH with those which have been observed following operation

MATERIALS AND METHODS

Observations were made of peripheral blood platelet count eosinophil count and patterns of megakaryocyte activity before during and after administration of ACTH to adult volunteers Four different therapeutic plans were used

Group I – (3 subjects) – a total of 200 mg of ACTH intramuscularly in divided doses (45 mg 45 mg 30 mg 30 mg 25 mg 25 mg) given twice daily for 3 days

Group II – (2 subjects) – a single intramuscular injection of 80 mg of ACTH gel

Group III – (5 subjects) – a 4 hour intravenous infusion of 5 mg of ACTH in 960 cc of 5 per cent dextrose solution

Group IV – (3 subjects) – control group same as in III, except ACTH omitted

Platelet counts and absolute eosinophil counts were done on venipuncture samples the former by the direct method using modified Rees Ecker diluting fluid[1] the latter by the technique of Henneman and associates[3]

Patterns of megakaryocyte activity were studied in stained smears of bone marrow obtained by needle aspirations of vertebral spinous processes in upper lumbar or lower thoracic region Activity profiles were formulated from a scheme of differential classification described in a previous communication In this scheme the number of platelets in a cell of this series is taken as an index of current productivity of that cell as long as there remains some cytoplasm not as yet converted to platelets A designates cells containing no platelets B 1 to 10 platelets C more than 10 platelets Cells whose extranuclear substance is exclusively platelets are classified as D forms the percentage representation of these defunct platelet laden units presumably reflects past production and the present rate of release of platelets into the blood stream

RESULTS

The data are presented graphically in Figure 1 and Figure 2

*From Veterans Administration Hospital West Roxbury Massachusetts

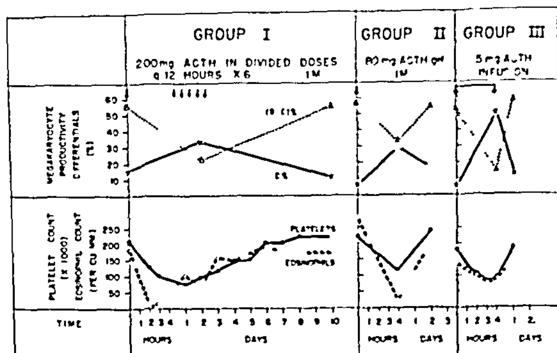


Fig 1 The effect of ACTH on eosinophil and peripheral platelet levels and on megakaryocyte productivity The means of the data in each group are represented The responses indicated for the subjects of Group III are identical with the responses which have been observed during the early period following major surgery

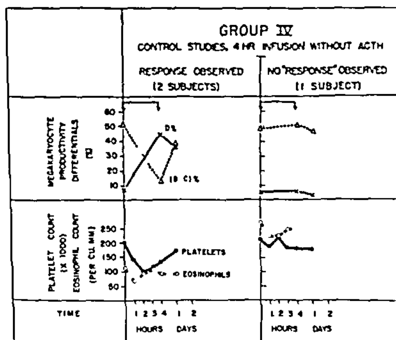


Fig 2 The effect of a 4 hour infusion of Dextrose solution (not containing ACTH) on eosinophils platelets and megakaryocytes One subject showed no changes Each of the other 2 developed marked changes which were similar to those observed in Group III

In Groups I II and III the early postoperative thrombocytopenia and eosinopenia were simulated in all subjects during hormone therapy Counts returned to pre ACTH levels within 2 days following treatment A late thrombocytosis as frequently occurs in the second week following operation was not observed

The induced thrombocytopenia was invariably accompanied by altered

patterns of megakaryocyte activity. In each instance these changes resembled in kind the responses to surgery; the degree to which they progressed appeared to be a function of the dosage plan employed. Those who received repeated intramuscular injections of ACTH (Group I) and those who were given a single dose of the long acting gel preparation (Group II) showed less pronounced increases in the percentage of platelet laden D forms than has been found in surgical patients. The marrow alterations observed in Group III on completion of the slow infusion of ACTH were identical with the early response to operation.

Prompt return to pre-ACTH appearances was evident in all marrow smears following cessation of therapy.

A disconcerting surprise was experienced when it was found that 2 of the 3 individuals in Group IV (the "control" group receiving no ACTH) showed responses similar to the subjects receiving ACTH. The third member of this group showed no such changes. In the first 2 subjects eosinopenia developed. This was not observed in the third.

DISCUSSION

Available evidence[4, 5] justifies the assumption that maximal or nearly maximal adrenocortical secretion was induced in all subjects who received ACTH. There is at present no satisfactory explanation for the apparent differences in the marrow responses of Group I, II and III. Since these differences were quantitative rather than qualitative it is possible that they may be the result of variations in the rate of adrenocortical activation. The findings suggest that a slow intravenous infusion of ACTH simulates most closely the physiologic endocrine reactions to surgery.

The fall in eosinophils which occurred in 2 of the "controls" implied that the platelet and megakaryocyte changes observed in these 2 (both of whom were negroes) were also of adrenal origin. Group IV served as a "control" only in the negative sense that there was no adrenocortical stimulation by exogenous ACTH. The importance of emotional influences (fear and anxiety) in evoking the general alarm reaction is well recognized.[6] It is possible that such an endogenous mechanism underlies these "spontaneous" responses.

In no instance has an alteration in the peripheral platelet count been unaccompanied by characteristic alterations in megakaryocyte activity. As discussed in the antecedent study, we have concluded that these invariably concurrent responses are not interrelated as cause and effect but appear to be effects of a common cause.

SUMMARY

1. Observations were made of peripheral blood platelet count, eosinophil count and megakaryocyte activity in volunteer subjects before, during and after administration of ACTH by several different dosage plans.

2. The thrombocytopenia and eosinopenia typical of the early period following major surgery were simulated in all subjects and were invariably accompanied by characteristic alterations in megakaryocyte activity.

3. The nature of the observed alterations in megakaryocyte activity was in all instances similar to the early response to surgery.

4. The magnitude of the induced alterations in megakaryocyte activity

appeared to be a function of the ACTH dosage plan employed; the marrow changes following a 4 hour intravenous infusion of 5 mg. of ACTH were identical with the early response to surgery.

5. Platelet counts, eosinophil counts, and patterns of megakaryocyte activity rapidly returned to pre-ACTH appearances following completion of therapy.

6. Similar platelet and marrow alterations were developed by 2 of 3 volunteers who received a 4 hour intravenous infusion not containing ACTH; the eosinopenia which was also observed in these 2 individuals suggests that adrenocortical stimulation by endogenous mechanisms may have occurred.

7. These studies indicate that adrenocortical activity may be the major determinant in the eosinopenia, the thrombocytopenia and the altered patterns of megakaryocyte activity which characterize the early phase of the response to surgery.

REFERENCES

1. Warren, R., Lauridsen, J., and Belko, J. S.: Alterations in numbers of circulating platelets following surgical operation and administration of adrenocorticotropic hormone. Circulation, N. Y. 7: 481, 1953.
2. Williams, J. A., Belko, J. S., and Warren, R.: Thrombocytogenesis in surgical patients. Circul. Res. N. Y. [illegible]
3. Henneman, P. H., Wexler, H., and Westenhaver, M. M.: A comparison of eosin-acetone, phloxine-propylene glycol diluents in eosinophil counts. J. Laborat. Clin. M. 34: 1017, 1949.
4. Thorn, G. W., Jenkins, D., Laidlaw, J. C., Goetz, F. C., Dingman, J. F., Arons, W. L., Streeten, D. H. P., and McCracken, B. H.: Pharmacologic aspects of adrenocortical steroids and ACTH in man. N. England J. M. [illegible]: [illegible]88, 1953.
5. Thorn, G. W., Jenkins, D., and Laidlaw, J. C.: The adrenal response to stress in man. In Recent Progress in Hormone Research, Vol. 8, Chapt. 7. G. Pincus, ed. New York: Academic Press, 1953.
6. Selye, H.: The physiology and pathology of exposure to stress. ed. 1. Montreal, Canada: Acta Inc. Publisher, 1950, p. 31.

HYPOTENSION A COMPARISON OF THE DIFFERENCES IN THE HUMAN CEREBRAL HEMODYNAMIC AND METABOLIC RESPONSE TO DRUG INDUCED AND ACUTE HEMORRHAGIC HYPOTENSION*

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Hypotension continues to be a problem of great practical importance Everyone associated with the care of the surgical patient will at one time or another be confronted with a serious hypotensive problem The question which must be asked is how safe is hypotension? Efforts are being made to answer this question by direct quantitative investigations Measurements of critical organ blood flow during hypotensive periods have contributed valuable information Studies of cerebral hemodynamics during acute drug induced hypotension have revealed that the brain has an amazing adaptability to lowered blood pressures Cerebral blood flow is rather well maintained during drug induced hypotension providing the reduction in pressure head does not exceed the critical pressure for the individual[1,2] Maintenance of blood flow is effected in these instances by an adequate degree of cerebral vasodilatation[1] Recent investigations of the effect of acute hemorrhagic shock on cerebral hemodynamics[3] indicate that there is a fundamental difference between the cerebral response to drug induced hypotension and the hypotension resulting from acute blood loss These differences are of great clinical importance for they demonstrate that the relative safety of hypotension as regards the brain is definitely related to the etiology of the hypotension

This study compares the hemodynamic and metabolic response of the human brain to drug induced hypotension and early acute hemorrhagic shock

METHOD

Twenty patients provided the material for this study Ten patients were made hypotensive by the intravenous injection of hexamethonium bromide in doses sufficient to produce a maximum fall in blood pressure Ten patients were made hypotensive by the removal of sufficient quantities of blood until signs and symptoms of early hemorrhagic shock appeared All studies were performed in an operating room under closely supervised conditions Each patient was checked constantly by continuous electrocardiograph and direct femoral arterial blood pressure monitoring Mean pressure was calculated by planimetry

With the unpremedicated patient in the supine position a control cerebral blood flow (C) determination was performed and calculated according to the original nitrous oxide method[4] Ten patients were then made hypotensive by intravenous injections of hexamethonium bromide in

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Table 1 Cerebral Hemodynamics in Hexamethonium Induced Hypotension

(MEAN VALUES 10 PATIENTS)	CONTROL	HYPOTENSION
MABP mm Hg	117	62†
CBF cc /100gm /min	53	47
CVR mm Hg/cc /100gm /min	2.4	1.3†
CMR cc O /100gm /min	3.3	3.4

†Statistically significant ($p<0.01$)

sufficient dosage to produce a maximum fall in blood pressure In every case a blood pressure floor was reached below which it was impossible to reduce the pressure no matter how much drug was injected In 5 of the 10 patients to whom hexamethonium was administered the head of the table was tilted upward 15 degrees in an effort to produce a greater blood pressure reduction With the blood pressure stabilized at the maximum hypotensive level a second cerebral blood flow determination was made (F)

In the second group of 10 patients following a control blood flow (C) determination each subject was then slowly but progressively bled via the femoral artery until signs and symptoms of hemorrhagic shock appeared These findings consisted of mental changes (apprehension confusion restlessness irritability excitement nausea and vomiting) generalized perspiration pilomotor changes cold moist skin increased rate and depth of respiration and hypotension While the signs and symptoms of hemorrhagic shock actively persisted with hypotension a second cerebral blood flow determination (L) was made Arterial and internal jugular blood samples were analyzed for oxygen and carbon dioxide values using the manometric methods of Van Slyke[5] pH determinations were made anaerobically in a glass electrode and corrected for temperature by suitable factors[6]

RESULTS

1 The Effect of Hexamethonium Bromide and Head up Tilt With a fall in mean arterial blood pressure from 117 to 62 mm Hg (47 per cent fall) cerebral blood flow was only reduced 12 per cent (53 to 47 cc /100 gm /min) (Table 1) This was not a statistically significant change Maintenance of cerebral blood flow was effected by an adequate degree (46 per cent of reduction in cerebral vascular resistance 2.4 to 1.3 resistance units) which represents a statistically significant change Cerebral oxygen consumption during hexamethonium induced hypotension was unaffected (3.3 to 3.4 cc /100 gm /min) Oxygen content fell uniformly in both arterial and internal jugular blood during hypotension without a significant change in A V oxygen difference (Table 2)

2 A The Effect of Hypovolemic Hypotension Without Hemorrhagic Shock (Table 3) A reduction in mean arterial blood pressure of 16 per cent (102 to 55 mm /Hg) produced by the removal of blood failed to reduce cerebral blood flow (13 to 17 cc /100 gm /min) Blood flow was effectively maintained by a significant decrease (55 per cent) in cerebral vascular tone (2 4 to 1 1 resistance units) This was similar to the response produced by the intravenous injection of hexamethonium However during hemorrhagic hypotension cerebral metabolism increased 21 per cent (2 9 to 3 7 cc / 100 gm /min)

B The Effect of Early Acute Hemorrhagic Shock (Table 3) A reduction in mean arterial blood pressure of 53 per cent (102 to 18 mm /Hg) produced by the withdrawal of blood resulted in a significant decrease in cerebral blood flow of 26 per cent (13 to 32 cc /100 gm /min) The failure to maintain cerebral blood flow was due to an inadequate degree of cerebral

Table 2 The Effect of Hypotension Induced by Hexamethonium and Head up Tilt

(MEAN VALUES 10 PATIENTS) BLOOD CONSTITUENTS	CONTROL	HYPOTENSION
ARTERIAL		
O_2 content vol %	15 8	14 7†
CO_2 content vol %	49 4	50
pH	7 41	7 41
pCO mm Hg	10	41
Mean arterial B P	117	62†
A V O_2 difference	6 3	7.3
INT JUGULAR		
O_2 content vol %	9.5	7 4†
CO_2 content vol %	55 4	57.3†
pH	7.37	7.36
pCO_2 mm Hg	50	52
A V CO_2 difference	6 0	7 4

†Statistically significant ($p<0.01$)

Table 3 Cerebral Hemodynamics in Hemorrhagic Shock

	CONTROL	HYPOTENSION	SHOCK
MABP mm Hg	102	55†	48†
CBF cc /100gm /min	43	47	32†
CVR mm Hg/cc /100gm /min	2 4	1 1†	1 6*
CMR cc O_2/100gm /min	2 9	3 7	3 6

Mean results of 10 patients rendered hypotensive by acute blood loss
Statistically significant †$p < 0.01$ *$p < 0.05$

Table 4 The Effects of Hypovolemic Hypotension and Hemorrhagic Shock

BLOOD CONSTITUENTS	CONTROL	HEMORRHAGIC HYPOTENSION	HEMORRHAGIC SHOCK
ARTERIAL			
O_2 content vol %	17.6	15.6	16.8
CO_2 content vol %	47.3	42.9	36.9†
pH	7.42	7.39	7.51†
pCO_2 mm Hg	38	36	25†
Mean arterial B.P.	102	[illegible]†	48†
A-V O_2 difference	6.8	7.8	11.5†
INT. JUGULAR			
O_2 content vol %	10.9	7.8	5.1†
CO_2 content vol %	55.6	50.4	49.3
pH	7.37	7.33	7.41
pCO_2 mm Hg	48	48	40
A-V CO_2 difference	6.4	8.1	12.4†

†Statistically significant ($p < 0.01$)

vascular dilatation (31 per cent decrease in cerebral vascular resistance — 2.1 to 1.6 resistance units). This was perhaps due to the significant decrease in carbon dioxide tension (38 to 25 mm./Hg) (Table 4), and the significant rise in arterial pH (7.42 to 7.51) which was observed during early acute hemorrhagic shock. Oxygen consumption increased during hemorrhagic shock (2.9 to 3.6 cc./100 gm./min.). Greater amounts of oxygen were extracted from arterial blood as evidenced by the significant decrease in venous oxygen content (10.9 to 5.1 volumes per cent) and significant increase in A-V oxygen difference (6.8 to 11.5 volumes per cent) (Table 4).

DISCUSSION

During periods of hypotension the brain attempts to maintain the constancy of its circulation by an adequate and proportionate degree of cerebral vascular dilatation. Evidence is available[7,8,9] which demonstrates that this response is a non-specific one and that the stimulus for cerebral vascular dilatation is hypotension itself regardless of the etiology of the hypotension. There is also evidence available which indicates that the intensity of this stimulus is dependent upon the degree of hypotension[5] i.e. — the greater the magnitude of the decrease in mean arterial blood pressure the greater will be the degree of cerebral vascular dilatation. Obviously an end point in hypotension must be reached beyond which cerebral circulation will fail because the fall in pressure head exceeds the dilating capacity of the cerebral vessels. In such cases cerebral vascular insufficiency results. These critically low pressures were not reached in this study. The results of this investigation demonstrated a significant difference between the cerebral hemodynamic response to drug induced hypotension and hypotension resulting from acute blood loss. While cerebral blood flow was well maintained during hexamethonium induced hypotension with no change in cerebral oxygen consumption and hypotension resulting from moderate blood loss, such was not the case when hypotension was part of the early acute hemorrhagic shock picture. Hyperventilation of unknown etiology is a constant finding in the early phases of acute hemorrhagic shock. A sharp reduction in the carbon

dioxide tension of arterial blood and a rise in arterial pH will result and were observed in this study Carbon dioxide is a potent cerebral vasodilator[10] Low carbon dioxide tensions produce cerebral vasoconstriction with an increase in vascular resistance[11] In the present study hypotension produced by the withdrawal of blood but without the signs and symptoms of shock and without producing hyperventilation was well tolerated by the brain with the maintenance of cerebral circulation However with the onset of the shock syndrome and hyperventilation normal cerebral compensation could not occur While some decrease in cerebral vascular tone did result (2 1 to 1 6 resistance units) this degree of cerebral vasodilatation was not sufficient to maintain cerebral circulation Low carbon dioxide tension prevented a more complete degree of cerebral vascular dilatation It appears that changes occur in the early phases of acute hemorrhagic shock which act to the detriment of the organism certainly in terms of cerebral hemodynamics This is a significant difference from the response of the brain to drug induced hypotension or hypotension resulting from moderate blood loss without shock symptoms This factor should be seriously considered by those proponents of arteriotomy as a method of controlling bleeding during surgery

SUMMARY

1 Cerebral hemodynamic and metabolic studies were performed on 10 patients made hypotensive by the intravenous injection of hexamethonium bromide A 17 per cent decrease in mean arterial blood pressure did not produce a significant reduction in cerebral blood flow Blood flow was maintained by a 46 per cent reduction in cerebral vascular resistance Cerebral oxygen consumption did not change

2 Cerebral hemodynamics and metabolic studies were performed on a second series of 10 patients made hypotensive by the withdrawal of blood With a decrease in mean blood pressure of 16 per cent without any evidence of true hemorrhagic shock and hyperventilation cerebral blood flow increased slightly due to a significant decrease in cerebral vascular resistance (55 per cent) Cerebral metabolism increased however

3 When early acute hemorrhagic shock appeared following the withdrawal of larger amounts of blood a fall in mean pressure of 53 per cent resulted in a significant fall in cerebral blood flow of 26 per cent The failure of the brain to compensate adequately was due to an inadequate degree of cerebral vascular dilatation (31 per cent decrease) Restriction of cerebral vascular dilatation was the result of a respiratory alkalosis secondary to hyperventilation of unknown etiology

4 The response of the brain to acute reductions in mean pressure is more complete when hypotension is not the result of acute hemorrhagic shock During early acute hemorrhagic shock factors operate which greatly restrict the adequacy of the various cerebral compensating mechanisms and result in a significant reduction of cerebral circulation in the face of increased cerebral oxygen demand and utilization

REFERENCES

1 Stone H H Mackrell T N and Wechsler R The effect on cerebral circulation and metabolism in man of acute reductions in blood pressure by means of intravenous hexamethonium bromide and head up tilt Anesthesiology *16* 168 176 1955

2 Moyer J H and Morris C Cerebral hemodynamics during controlled hypotension induced by the continous infusion of ganglionic blocking agents (hexamethonium pendiomide and arfonad) J Clin Invest 33 1081 1088 1954

3 Stone H H, Mackrell T N, Brandstater B J Haidak C and Nemir P The effect of induced hemorrhagic shock on the cerebral circulation and metabolism of man in Surgical Forum 1953 Philadelphia W B Saunders Co 1954 pp 789 791

4 Kety S S and Schmidt C F The nitrous oxide method for the quantitative determination of cerebral blood flow in man theory procedure and normal values J Clin Invest, 27 476 483 1948

5 Peters J P and Van Slyke D D Quantitative Clinical Chemistry Vol II Baltimore Williams and Wilkins Co 1932

6 Rosenthal T B The effect of temperature on the pH of blood and plasma *in vitro* J Biol Chem, *173* 25 30 1948

7 Morris C C, Jr, Moyer J H, Snyder H C and Haynes C W Jr Vascular dynamics in controlled hypotension a study of cerebral and renal hemodynamics and blood volume changes Ann Surg, *135* 706 1953

8 Shenkin H A Effects of various drugs upon cerebral circulation and metabolism in man J Appl Physiol, *3* 465 1951

9 Hafkenschiel J H, Crumpton C W, Moyer J H and Jeffers W A The effects of dihydroergocornine on the cerebral circulation of patients with essential hypertension J Clin Invest, 29 408 1950

10 Kety S S and Schmidt C F. The effects of altered arterial tensions of carbon dioxide and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young men J Clin Invest, 27 484-492 1948

11 Kety S S and Schmidt C F The effects of active and passive hyperventilation on cerebral blood flow cerebral oxygen consumption cardiac output and blood pressure of normal young men J Clin Invest, 25 107 119 1946

Problems in Hypothermia and Intracardiac Surgery

INTRODUCTION

Richard L. Varco

Among the investigators presenting papers at the Forum on Fundamental Surgical Problems interest remains keen in the research intimately related to the heart and great vessels. This vein of new information broached by those workers in the laboratory and in the clinics continues to supply a provocative yield. With heart disease the rampant killer nationally, continued progress in this area is essential if we are ever to turn this trend downward or even slow its rate of upward ascent.

Creech and his associates have investigated the vulnerability of canine aortic homografts to induced severity hypercholesterolemia and have been able to demonstrate an increased susceptibility in the graft to atherosclerotic changes and at a serum cholesterol level below that which will produce comparable widespread systemic arteriosclerotic change. Their proposal to expand this study to textile prostheses has much to recommend it. Apropos of the considerable need for accurate localization of arteriosclerotic obstructive lesions of the coronary arteries as an important facet of the atherosclerosis problem, Cannon and co-workers reported on a method for coronary arteriography in the dog. They were able to obtain excellent angiograms by momentarily occluding aortic flow via a balloon attached to a cannula which had been inserted down the aorta. After the injection of radiopaque dye *into the limited supravalvular compartment* an accurate outline of the coronary circulation could be obtained roentgenographically. A comparison of these pictures together with those obtained after ligation of a coronary artery and finally with control preparations obtained by sacrificing the animal showed excellent agreement. Once this method can safely be adapted to clinical material, the precise information thus revealed and not otherwise readily obtainable will be invaluable in documenting those cases most appropriate for surgical relief of their vascular insufficiency.

The handicap of an increased incidence of ventricular fibrillation under hypothermia appears to be susceptible of better control through a variety of approaches. Hufnagel and his associates report on the effectiveness of intravenous prostigmin combined with sinoauricular nodal block by xylocaine as a means of preventing ventricular fibrillation in dogs whose temperature has been reduced below 26 C. Shumacker in the discussion confirmed the advantages of this approach as tested in their laboratory. Baer *et al.*, find that neostigmin also reduces the incidence of ventricular fibrillation after ventriculostomy in animals hypothermic to 26 C. when this material is administered by coronary perfusion prior to the onset of

caval inflow stasis. Lewis and Niazi have been able to abolish, virtually, the incidence of ventricular fibrillation despite cooling of their dogs to below 10 C. if these animals are ventilated with 5 to 10 per cent carbon dioxide in oxygen during the period of induced hypothermia. The addition of a right ventriculotomy to adult dogs with comparably reduced body temperatures failed to produce ventricular fibrillation in any instance. They also report that this technique has been applied with success to a series of clinical cases.

Cates and associates from the Mayo Clinic describe 2 variations of the Vineberg maneuver for vascularizing the myocardium — methods in which the experimental animal will tolerate high ligation of the anterior descending coronary artery more frequently than will the unprotected control group. They tested a preparation with flow through the internal mammary artery permitting it to drain initially into the left auricle as well as via its divided intercostals into the myocardium. They report that anastomotic channels were then established between the graft vessel, its branches and the surrounding muscular syncitium with a minimal tendency to obliterative changes in the implanted artery. Following ligation of the nutrient anterior descending artery, approximately half of the dogs would survive. This figure, while yielding recovery rates greater than for the control group, was in turn exceeded in percentage of those surviving when the subclavian artery was used for the source of supplemental blood supply.

POTASSIUM EFFECTS IN HYPOTHERMIA *

An Experimental Study

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There is some evidence that the myocardium loses potassium during the induction of general hypothermia to 27° C [1-6] If circulatory arrest is undertaken by caval occlusion this trend is accentuated[4] Furthermore during ventricular fibrillation potassium leaves the heart in considerable quantities[3,7] In theory protection of the heart during caval occlusion would entail marked slowing or arrest the latter having considerable advantages as regards attendant cardiac surgery As potassium will conveniently depress all heart activity[5], it would seem rational to use it to cause arrest during caval occlusion and in the presence of ventricular fibrillation provided resuscitation is feasible for its administration might achieve correction of the myocardial cell deficit

In this study the effects of the administration of potassium to the hypothermic heart have been investigated both in the treatment of ventricular fibrillation and in the induction of cardiac arrest This report is also concerned with a satisfactory method of cardiac resuscitation from potassium arrest

METHOD

In 14 fasting (overnight) mongrel dogs of an average body weight of 17 kg hypothermia was induced by water bath surface cooling to a rectal temperature of 26 C Pentothal sodium anesthesia was used in all cases the initial average dose being 16 mg/kg This drug was also used to maintain a light anesthesia before surface cooling and to control shivering during cooling the latter required 50 to 100 mg on each occasion The average total dosage was 700 mg After endotracheal intubation respirations were controlled at the rate of 24 per min using oxygen at 500 ml per min During cooling which averaged 35 min for the 14 dogs a CO_2 absorber was added to the closed circuit Blood samples were removed for arterial CO estimations throughout the experiment

After removal from the water bath a right thoracotomy was carried out with mobilization and control of the venae cavae and ligation of the vena azygos Thereafter the pericardium was opened and control of the ascending aorta established During the operative procedure which required 50 to 60 min the average temperature drift was 3 3°C After resuscitation rewarming was carried out to 37°C in a hot water bath at 47°C

Polyethylene catheters had been previously inserted via the left femoral artery and vein the former being threaded to lie in the ascending aorta the latter in the inferior vena cava Arterial and venous pressures were recorded by means of 2 Statham strain gauges connected to a continuous Sanborn poly viso 4 channel recorder Via the appropriate catheter 100 cc of heparinized whole blood were given intra arterially at the end of the period of inflow tract occlusion and 50 per cent glucose and crystalline insulin intravenously during resuscitation Ten cc of 1 per cent protamine

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sulphate was administered intravenously during chest closure. Arterial samples were taken for plasma potassium and glucose levels.

Intermittent electrocardiographic recordings were taken throughout each experiment with right foreleg, right and left hind leg leads.

Potassium was administered as KCl solution 1 mEq./ml. at pH 5.6. With occlusion of the inflow tracts the solution of KCl was injected into the first part of the ascending aorta which had been clamped distally. In this way perfusion of the coronary arterial system was obtained aided by cardiac massage. The KCl solution was slowly injected in sufficient quantity to cause complete electrocardiographic arrest.

POTASSIUM ADMINISTRATION

The aim of potassium administration was to produce electrocardiographic arrest in the presence of either sinus rhythm or ventricular fibrillation. Clinical observation of cardiac arrest was not accurate and if total depression of all myocardial functions did not occur, portions of the ventricles escaped and served as foci for ectopic activity so that wave fibrillation resulted soon after massage was instituted.[4] The sequence of events following the institution of massage after potassium arrest have been described recently.[5] In most cases after a period of potassium asystole during which there was marked improvement in myocardial color and tone and a rise in the arterial pressure, a responsive phase arose when the ventricles contracted only after the stimulus of touch or of massage particularly of the septal area. The right atrium at this time usually showed ripple-like contractions, the result of backspread of the ventricular impulse.

The ensuing course of events was variable. Most often in the absence of atrial contractions of sinus origin idioventricular rhythm developed. Thereafter there was a gradual return of good atrial contractions and heart block preceded the development of sinus rhythm. On some occasions recovery was compromised by wave fibrillation which could only be abolished by a second and often larger dose of potassium. This cycle of potassium arrest and massage was carried out on occasion 5 or 6 times before successful resuscitation was obtained.

In 14 dogs the average dose of potassium achieving arrest was 7.9 mEq. The largest single dose was 28 mEq. and the largest total dose 80 mEq. Twenty-five dogs received more than one dose. In 10 dogs potassium arrest followed ventricular fibrillation of varying periods and in the remainder it was induced in the presence of sinus rhythm (Table 1.) The heart was frequently restarted in the presence of plasma potassium levels in the range of 6.2 to 8.4 mEq./L. The greatest danger of very large potassium doses lay in their late effect on the resuscitated heart due to high plasma potassium levels. In such cases the deterioration of heart action could be followed on the electrocardiogram and was associated with falling arterial and rising venous pressures. By the use of 50 per cent glucose and insulin the high plasma potassium level could be controlled and possible recurrence of cardiac arrest averted.

RESUSCITATION

In all cases resuscitation was commenced by massage immediately after the release of inflow tract occlusion. It became increasingly apparent that with massage alone after potassium arrest recovery, although finally success-

Table 1. Details of Potassium Arrest, Caval Occlusion and Heart Recovery

POTASSIUM ARREST TIME OF ARREST	CAVAL OCCLUSION TIME AND INTERVAL TO EFFECTIVE MASSAGE PRESSURE						HEART RECOVERY TO SINUS RHYTHM AND NORMAL EKG CAVAL OCCLUSION TIME		
	Less than 20 min			More than 20 min					
NO. OF DOGS	1-5	6-10	11-20	20-25	26-30	30	Less than 20 min	More than 20 min	Total
At outset of caval occlusion									
25	3	5	9	1	3	1			
	Total 17			Total 8			17	6	25
At end of caval occlusion									
9			7	2					
	Total 7			Total 2			7†	2	9
With ventricular fibrillation									
10	1	4	5						
	Total 10						10		10

*Includes 10 auriculotomies and 5 ventriculotomies
†Includes 3 auriculotomies

ful took on occasion a prohibitively long time and sometimes included multiple doses of potassium. Although the concentration of potassium in the extracellular tissues of the heart could be lowered slowly by massage, a more rapid method of eliminating the excess potassium was necessary. Large doses of hypertonic glucose were used in the belief that the concentration of potassium might be decreased by the osmotic effect of the solution. Furthermore, the possibility existed that glucose might lower the plasma potassium level as it entered skeletal muscle and also aid replenishment of the myocardium. In 22 cases doses of 20 ml. of 50 per cent glucose were administered intravenously as part of the recovery regime, crystalline insulin being used along with glucose in 13 cases.

Fifty per cent glucose has produced restoration of spontaneous idioventricular rhythm and of sinus activity in cases where idioventricular rhythm was already present. It has also produced coordination of ventricular activity and converted potassium induced "wave" fibrillation to an effective ventricular beat. Electrocardiographic changes have been the elimination of features associated with a raised plasma potassium level and the absence from the recovery pattern of abnormal QRS complexes of high amplitude.

However, to be most effective glucose had to be given when the ventricles were in the "responsive" phase. Given at this time its effects always proved dramatic and recovery of spontaneous ventricular contractions occurred in every case. Thirteen dogs have been so treated, each successfully as regards cardiac resuscitation and ultimate survival. In this group ventricular and electrocardiograph recovery times were significantly shortened (Table 2). If glucose was given at any time other than when the ventricles were "responsive," no great improvement in recovery times was noted.

Of 31 dogs with periods of cerebral anoxia under 20 min., so that survival was expected, 6 died of pulmonary complications within 18 hours

Table 2 Ventricular and EKG Recovery Time after Potassium Arrest with and without 50% Glucose

	SINGLE K DOSE WITH ARREST	MULTIPLE K DOSES (RECOVERY FROM LAST ARREST)	TOTAL AND AVERAGE
WITH GLUCOSE	9 DOGS	4 DOGS	13 DOGS
Ventricular Recovery Time			
Average	8.5 min	1[illegible] min	10.4 min
Range	4 to 12 min	8 to 20 min	4 to 30 min
EKG Recovery Time			
Average	16.3 min	22.5 min	17.8 min
Range	8 to 40 min	17 to 30 min	8 to 40 min
WITHOUT GLUCOSE	9 DOGS	3 DOGS	12 DOGS
Ventricular Recovery Time			
Average	13.5 min	22.7 min	1[illegible].8 min
Range	15 to 30 min	10 to 34 min	[illegible] to 34 min
EKG Recovery Time			
Average	17.6 min	68 min	[illegible]2.8 min
Range	9 to 81 min	43 to 95 min	9.0 to 95 min

Note.—Ventricular Recovery Time is the time from the end of caval occlusion until the cessation of massage and the first spontaneous ventricular contractions capable of maintaining an arterial pressure of 50 mm Hg. FKC Recovery Time is from the end of caval occlusion to an electrocardiogram showing sinus rhythm and approaching normality as regards the control taken before cooling.

DISCUSSION

Potassium will produce complete depression of myocardial irritability and conductivity. Not only large doses but high concentrations are required to accomplish these effects. As electrocardiographic arrest is the required aim of potassium administration, it is safer to err on the side of too large a dose than too small. The former will only slightly prolong the period of ventricular asystole; the latter will compromise resuscitation.

The vasopressor action of arterially injected potassium is the result of several mechanisms. Its occurrence at the outset of massage greatly facilitates oxygenation of the myocardium. The increased myocardial tone at this time may indicate that potassium is entering the myocardial cell, as a similar phenomenon occurs in smooth muscle.

With manual control of the heart, a striking feature of this study has been the tolerance of the heart to large doses of potassium. On occasion small doses of potassium, although causing electrocardiographic arrest, did not produce a "responsive phase," and only after a much larger dose had been given did the heart improve in color and tone, becoming "responsive," and recovering well thereafter. It is difficult to explain why a "responsive phase" should arise unless it represents potassium replenishment of the myocardial cell. If this is the case, the concentration of the excess extracellular potassium may be satisfactorily reduced by the osmotic effect of a hypertonic solution. There are many advantages in using 50 per cent glucose for this purpose, not the least being the reduction of a high plasma

potassium level should this arise as potassium enters skeletal muscle in association with glucose Insulin will potentiate this movement[2] and may be necessary in hypothermia as there is some evidence that the utilization of glucose is impaired at low temperatures[4] Although with the doses of potassium used in this investigation difficulty has seldom arisen from high plasma potassium levels there is no doubt that should very large doses be utilized the plasma potassium level can be easily controlled and deterioration of cardiac action prevented by the judicious use of further glucose and insulin

By the method described the correction of ventricular fibrillation has not proven a problem Protection of the heart by caval occlusion to prevent distension is advisable and also early potassium arrest to forestall the continuing loss of potassium from which the fibrillating heart suffers

The successful use of potassium and glucose in resuscitation carries with it implications of the possible use of these substances in protecting the heart during cooling and caval occlusion This may possibly be unrewarding because of the inevitable effects of cold on the myocardium among them the probable impairment of glucose utilization During caval occlusion the effects of hypoxia are an added complication There is moreover no definite evidence in this study that potassium re-enters the myocardial cell during resuscitation although the overall picture makes it an interesting possibility

SUMMARY

1 Under general hypothermia 11 dogs were subjected to potassium induced cardiac arrest associated with varying periods of inflow tract occlusion Details are given of the effects of potassium perfusion of the hypothermic heart and the sequence of events following resuscitation by massage alone

2 A method of resuscitation from potassium arrest is described using 50 per cent glucose and insulin By such a method the return of spontaneous heart action and of a normal electrocardiogram has been significantly hastened

3 Induced potassium arrest at the outset of caval occlusion was carried out in 25 dogs 10 of which underwent right auriculotomy and 5 right ventriculotomy during the arrest

4 In 42 out of 44 dogs resuscitation to normal heart action was achieved

REFERENCES

1 Covino B G and Hegnauer A H Electrolytic and pH changes in relation to Hypothermic Ventricular Fibrillation In press

2 Fenn W O Potassium in Physiological Processes Physiol Rev *20* 377 1940

3 Kehar N D and Hooker D R Evidences of an Altered Tissue State in Ventricular Fibrillation Am J Physiol *112* 301 1935

4 Mavor G E Harder R A McEvoy R K McCoord A B and Mahoney E B Potassium and the Hypothermic Heart In press

5 Mavor G E Harder R A McEvoy R K and Mahoney E B The Administration of Potassium to the Hypothermic Heart In press

6 Olsen N S Rudolph G G and Gollon F Electrolytic Transfers in Plasma Skeletal Muscle and Heart of Normo and Hypothermic Dogs during Hyperventilation and Anoxia Fed Proc Balt *14* 108 1955

7 Swan H Zeavin I Blount S G and Virtue R W Surgery by Direct Vision in the Open Heart Under Hypothermia J Am M Ass *153* 12 1081 1953

8 Wiggers C J Studies on Ventricular Fibrillation Produced by Electric Shock The Action of Antagonistic Salts Am J Physiol *93* 197 1930

THE EFFECT OF LOWERED BODY TEMPERATURE ON THE CEREBRAL HEMODYNAMICS AND METABOLISM OF MAN*

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CELESTE DONNELLY AND ALFRED S FROBESE

It has long been recognized that reductions in body temperature produce an overall reduction in oxygen consumption and metabolism If body temperature is reduced sufficiently, it is possible to extend substantially the periods during which the arterial blood supply to the various critical body organs may be totally interrupted The recent broadened scope of cardiovascular surgery has necessitated in various instances total circulatory occlusion of sufficient duration to permit definitive surgical repairs In order to determine what constitutes a safe period of total circulatory interruption it is necessary to know the oxygen consumption of the critical organs at the specific level of hypothermia being employed The brain because of its great sensitivity to oxygen lack must receive prime consideration

Information has accumulated from both experimental and clinical studies indicating that at best at normal body temperature the human cerebral circulation may be interrupted for a 3 to 4 minute period with some degree of safety and without serious irreversible neurological damage If by the use of hypothermia the cerebral oxygen consumption is significantly reduced and this reduction is quantitatively measured then it might be possible to estimate how much longer the brain could safely tolerate total cessation of cerebral blood flow

The purpose of this study was to determine the quantitative changes in cerebral hemodynamics and metabolism of man occurring during generalized body cooling

METHOD

Studies were performed on 3 volunteer professional subjects Each subject was first well screened as to physical and mental health age and previous illnesses A complete history and physical examination with comprehensive laboratory studies constituted the work up of each subject

The studies were performed in an operating room under strictly controlled conditions A continuous electrocardiographic tracing was recorded throughout the study A needle inserted into the femoral artery was attached to a strain gauge manometer and recorder to obtain a continuous blood pressure tracing Mean pressure was calculated by planimetry Continuous rectal temperatures were recorded by means of a thermocouple

A control cerebral blood flow (c) determination was performed and calculated on each unpremedicated subject according to the original nitrous oxide method[1] Each subject then received an appropriate intravenous dose of a belladonna drug (atropine or scopolamine) and the nose and throat were well sprayed with cocaine (5 per cent) Following an intravenous dose of sodium pentothal a cuffed nasotracheal tube was inserted

*From the Departments of Anesthesiology and Surgery Graduate School of Medicine University of Pennsylvania and the University of Pennsylvania Graduate Hospital This study was aided by grants from the Wilson C Laucks Memorial Foundation the A Atwater Kent Fund and No H 1282 from the National Heart Institute of the National Institutes of Health Public Health Service

Coughing was controlled by sufficient amounts of sodium pentothal. A second cerebral blood flow (F 1) determination was made with the subject under moderate pentothal narcosis. The purpose of this second study was to establish the effect of sodium pentothal upon cerebral hemodynamics and metabolism and to be able to differentiate between the effects of pentothal and those changes produced by hypothermia.

The subjects were then placed in the conventional cooling blankets through which ice water was circulated by means of a pump. Anesthesia was maintained by cyclopropane and oxygen, administered through a closed to and fro system plus intermittent doses of sodium pentothal sufficient to control shivering. When rectal temperature fell to 81°F the cyclopropane was discontinued and after a waiting period of 20 minutes a third cerebral blood flow study was performed (F 2). Following the third flow, the subjects were rapidly warmed by circulating hot water through the blankets.

Arterial and internal jugular blood samples were analyzed for oxygen and carbon dioxide values using the Van Slyke manometric methods. pH determinations were made anaerobically in a glass electrode and corrected for temperature by suitable factors.[3] Values for carbon dioxide tension were calculated by the nomograms of Van Slyke and Sendroy.[4]

Table 1 The Effect of Reduced Body Temperature on the Cerebral Circulation and Metabolism of Man Patient #1 F 1 Age 21 Male Pentothal and Cyclopropane

	RESULTS			83 F
ARTERIAL	NORMAL MEAN	CONTROL	PENTOTHAL	HYPOTHERMIA
O_2 Content (Vol %)	18	20 5	[illegible]0 8	23 2
CO_2 Content (Vol %)	50	11 5	53 9	17 2
pH	7 42	7 12	7 31	7 [illegible]3
pCO_2 (mm Hg)	10	38	52	17
Mean Blood Pressure (mm Hg)	8[illegible]	82	82	89
A V Oxygen Difference	7	8 5	6 1	8
Hematocrit	15	12 6	19 2	52 8
INTERNAL JUGULAR				
O_2 Content (Vol %)	11	12	11 1	15 2
CO_2 Content (Vol %)	5	53 2	[illegible]	53 1
pH	7 37	7 37	7 30	7 19
pCO (mm Hg)	50	17	60	70
A V CO_2 Difference	5	8 7	1 3	6 2
CEREBRAL				
Blood Flow (cc /100 g/min)	51	13	41	12
CMR O_2 (cc /100 g/min)	3 3	3 7	2 8	0 9
Vascular Resistance (mm Hg/cc /100 g/min)	1 6	1 8	1 9	7 1

Table 2 The Effect of Reduced Body Temperature on the Cerebral Circulation and Metabolism of Man Patient #2 P H Age 42 Male Pentothal and Cyclopropane

ARTERIAL	RESULTS NORMAL MEAN	CONTROL	PENTOTHAL	84.8 F HYPOTHERMIA
O_2 Content (vol %)	18	[illegible]	[illegible]	[illegible]
CO_2 Content (vol %)	[illegible]	[illegible]	[illegible]	41.2
pH	7.42	7.19	[illegible]	[illegible]
pCO_2 (mm Hg)	40	[illegible]	[illegible]	[illegible]
Mean Blood Pressure (mm Hg)	[illegible]	107	[illegible]	111
A-V Oxygen Difference	7	7.1	[illegible]	8
Hematocrit	[illegible]	[illegible]	[illegible]	[illegible]
INTERNAL JUGULAR				
O_2 Content (vol %)	11	9	[illegible]	[illegible]
CO_2 Content (vol %)	[illegible]	[illegible]	[illegible]	[illegible]
pH	7.37	[illegible]	7.47	[illegible]
pCO_2 (mm Hg)	50	43	40	[illegible]
A-V CO_2 Difference	5	6.4	2.0	6.6
CEREBRAL				
Blood Flow cc./100 g/min	54	40	42	19
CMR O_2 cc./100 g/min	3.3	2.8	1.6	1.1
Vascular Resistance mm Hg/cc./100 g/min	1.6	2.5	2.2	5.7

RESULTS

The first subject (T E) (Table 1) was deeply anesthetized with 500 mg of nembutal sodium intravenously, 1 gm of pentothal sodium and cyclopropane. Though respiration did not cease even at temperatures of 79.2 F, gas exchange was not completely adequate. At a temperature of 83 F a respiratory acidosis had developed (arterial pH 7.42 to 7.23) (arterial CO_2 content 41.5 to 47.2 vol per cent) (arterial CO_2 tension 38 to 47 mm Hg). Internal jugular blood reflected similar changes (pH 7.37 to 7.19) (CO_2 tension 47 to 70 mm Hg). During hypothermia (83 F) cerebral blood flow decreased 72 per cent (43 to 12 cc./100 g/min), cerebral oxygen consumption decreased 76 per cent (3.7 to 0.9 cc./100 g/min) and cerebral vascular resistance increased 311 per cent (1.8 to 7.4 resistance units). The increase in resistance is a reflection of hemoconcentration with a 20 per cent increase in hematocrit (42.6 to 52.8) plus an advanced degree of generalized vasoconstriction. Shivering did not occur in this study.

The second subject (P H) (Table 2) was only lightly anesthetized (500 mg pentothal plus sufficient amounts of cyclopropane to control shivering). At the time of the third cerebral blood flow during moderate hypothermia (84.8 F) the general pattern of response noted in the first subject was repeated. In spite of assisted respiration respiratory acidosis developed as reflected in the arterial and venous changes in pH and CO_2 tension. Cerebral blood flow decreased 52 per cent (40 to 19 cc./100 g/min), cerebral oxygen consumption decreased 61 per cent (2.8 to 1.1 cc./100 g/min) while cerebral vascular resistance increased 128 per cent

Table 3. The Effect of Reduced Body Temperature on the Cerebral Circulation and Metabolism of Man. Patient #3 J. R. Age 32 Male Pentothal and Cyclopropane

ARTERIAL	RESULTS NORMAL MEAN	CONTROL	PENTOTHAL	82.6 F HYPOTHERMIA
O_2 Content (vol %)	18	17.1	17.0	22.9
CO_2 Content (vol %)	50	50.7	52.0	38.5
pH	7.42	7.44	7.33	7.24
pCO (mm Hg)	40	39	51	46
Mean Blood Pressure (mm Hg)	85	93	93	97
A V Oxygen Difference	7	3.6	4.0	8.5
Hematocrit	45	40.5	40.0	51.8
INTERNAL JUGULAR				
O_2 Content (vol %)	11	13.5	13.0	14.4
CO_2 Content (vol %)	55	55.8	56.6	47.1
pH	7.37	7.35	7.31	7.19
pCO_2 (mm Hg)	50	54	57	64
A V CO_2 Difference	5	5.1	4.6	8.6
CEREBRAL				
Blood Flow cc./100 g/min	54	50	51	44
CMR O_2 cc./100 g/min	3.3	1.8	2.2	3.7
Vascular Resistance mm Hg/cc./100 g/min	1.6	1.7	1.6	2.1

(2.5 to 5.7 resistance units). *Temperature was not reduced further in this* subject because of the development of many ventricular arrhythmias.

The third subject (J. R.) (Table 3) was lightly anesthetized with 850 mg. of pentothal sodium and cyclopropane. Shivering could not be adequately controlled by general anesthesia. Not desiring to introduce other variables into the study such as the use of curare drugs and controlled respiration, the third blood flow determination was performed at a temperature of 82.6 F with gross shivering present. This study demonstrated the effect of shivering on cerebral metabolism and hemodynamics. In spite of assisted respiration, a respiratory acidosis developed which was evident in the arterial and internal jugular blood samples (pH and CO tension). Cerebral blood flow fell only slightly (12 per cent) (50 to 44 cc./100 g./min.), cerebral oxygen consumption *increased* 105 per cent (1.8 to 3.7 cc./100 g./min.). Vascular resistance increased 23 per cent (1.7 to 2.1 resistance units), again reflecting hemoconcentration (hematocrit 40.5 to 51.8), and vasoconstriction.

DISCUSSION

The results of these studies demonstrate for the first time the quantitative response of the human brain to reductions in overall body temperature. As expected, cerebral metabolism and blood flow are reduced at lower temperature levels corresponding to the generalized decrease in body oxygen consumption which has been previously reported.[5, 6] However, because of the greater functional complexity of the brain and its

greater oxygen demands it is not valid to assume that at a constant lowered body temperature cerebral metabolism is depressed to the same degree as other body tissues Further studies of this type are needed before this relationship may be clarified

In these studies with shivering well controlled cerebral metabolism may be greatly reduced (76 per cent T E) with reductions in body temperature which have been clinically proven to be safe (83 F) This reduction in cerebral metabolism (26 per cent of cerebral oxygen consumption at normal body temperatures) at a temperature of 83 F compares quite favorably with the 85 per cent reduction in overall body oxygen consumption observed at a much lower temperature[7] (68°F)—a temperature in the human which produces serious cardiac complications It must be emphasized that the results obtained in this 1 study (T E) must not be used to predict a similar reduction in cerebral metabolism for all other patients whose body temperature is reduced to 83 F Depth of anesthesia previous metabolic rate and the presence of shivering will greatly influence the degree of metabolic depression at a particular hypothermic level

The third study (J R) demonstrates the effect of shivering on cerebral metabolism Even with a reduction in body temperature to 82 6 F cerebral oxygen utilization can be increased over 100 per cent in the face of a decrease in cerebral blood flow This disproportion between oxygen demand and supply could produce serious oxygen deficiencies of the brain It is particularly hazardous to assume that the mere reduction of body temperature implies a reduction in cerebral oxygen consumption The total interruption of the cerebral circulation beyond the clinically safe 3 to 4 minute period merely because hypothermia is being employed is dangerous unless shivering is completely controlled Others have emphasized the importance of shivering in increasing body metabolism[5, 8]

SUMMARY

1 Direct measurements of human cerebral hemodynamics and metabolism during periods of reduced body temperature were made in 3 subjects

2 The results obtained indicate that cerebral metabolism may be reduced 76 per cent when body temperature is reduced to 83°F providing shivering is controlled and anesthesia is deep (T E)

3 In the presence of shivering even at body temperatures of 82 6 F cerebral oxygen demand may be increased over 100 per cent The importance of shivering is discussed

4 Cerebral blood flow decreased in all 3 subjects with hypothermia levels

5 Cerebral vascular resistance in 2 subjects (T E and P H) increased 311 per cent and 128 per cent respectively This probably reflects a generalized vasoconstriction characteristic of the body response to hypothermia Hemoconcentration may also be a factor in the increase in vascular resistance

REFERENCES

1 Kety S S and Schmidt C F Nitrous oxide method for quantitative determination of cerebral blood flow in man theory procedure and normal values J Clin Invest 27 476-483 1948

2 Peters J P and Van Slyke D D Quantitative clinical chemistry Baltimore The Williams and Wilkins Co 1932 Vol 2
3 Rosenthal T B The effect of temperature on the pH of blood and plasma in vitro J Biol Chem *173* 25 30 1948
4 Van Slyke D D and Sendroy J Jr Studies of gas and electrolyte equilibria in blood line charts for graphic calculations by Henderson Hasselbalch equation and for calculating plasma carbon dioxide content from whole blood content J Biol Chem *79* 781 1928
5 Bigelow W G Lindsay W K Harrison R C Gordon R A and Greenwood W F Oxygen transport and utilization in dogs at low body temperatures Am J Physiol *160* 125 137 1950
6 Smith L W and Fay T Observation on human beings with cancer maintained at reduced temperatures of 75 to 90 F Am J Clin Path *10* 1 1940
7 Bigelow W G Callaghan J C and Hopps J A General hypothermia for experimental intracardiac surgery Ann Surg *132* 531 537 1950
8 Bigelow W G Lindsay W K and Greenwood W F Hypothermia Its possible role in cardiac surgery an investigation of factors governing survival in dogs at low body temperatures Ann Surg *132* 849 866 1950

THE USE OF CARBON DIOXIDE TO PREVENT VENTRICULAR FIBRILLATION DURING INTRACARDIAC SURGERY UNDER HYPOTHERMIA*

F JOHN LEWIS AND SUAD A NIAZI

The most serious complication associated with the use of hypothermia to perform open intracardiac surgery has been ventricular fibrillation All investigators who have used hypothermia encounter this complication Bigelow[1] lost one half of his animals due to fibrillation or standstill and Swan[7] somewhat later reported that all of a series of animals subjected to right ventriculotomy at temperatures below 25 C developed ventricular fibrillation We have had a lower incidence than this when operating on the ventricular septum in dogs[3] but in our hands too fibrillation has been a troubling complication both in animals and man Among the first 33 patients in whom we repaired atrial septal defects under direct vision ventricular fibrillation occurred in 11[4] Defibrillation both in dogs and man is usually possible with massage adrenalin and electrical shock but prevention is clearly a better alternative

Our previous experience has shown that it was possible by employing respiratory mixtures of carbon dioxide and oxygen under special circumstances to cool dogs to body temperatures below 10° C with survival[5,6] As a result of these experiments we have been led to use carbon dioxide while doing experimental intracardiac operations at temperatures below 20° C

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METHOD

Thirty adult mongrel dogs weighing 10 to 20 kg were cooled between refrigerating blankets and under nembutal anesthesia All of these dogs were adults with complete and mature permanent teeth The nembutal was given intravenously in an initial dose of 22 to 33 mg per kg of body weight This dosage was supplemented intermittently during cooling until a total dose of 55 to 77 mg per kg of body weight had been given by the end of the cooling period Cooling was usually stopped when the body temperature had dropped to 20° C but by the time the chest was opened and the circulation interrupted the temperature had fallen to between 17° and 18° C

Artificial respiration was started with the cooling and a mixture of 5 per cent carbon dioxide in oxygen was used for respiration This was changed to a 10 per cent concentration of carbon dioxide when the body temperature had reached 30 C and continued until the circulation was arrested A mechanical respirator which consisted of 2 solenoid valves operated by an electronic timer was used A respiratory rate of 10 per minute and a constant respiratory stroke volume were maintained during the experiment

The chest was opened in the right fourth interspace and the circulation was interrupted for 30 minutes by occluding the caval and azygos venous flow by means of umbilical tapes passed around these vessels When a ventriculotomy was added the right ventricle was opened longitudinally for a distance of 1 to 5 cm during the circulatory arrest The ventriculotomy wound was closed with 3 0 silk

Rewarming was done with the same blankets used for cooling but during rewarming a solution at 40° to 45° C was circulated through the blankets

The respiratory mixture of 10 per cent carbon dioxide in oxygen was continued during rewarming but finally changed to oxygen alone at a body temperature between 26° and 28 C Occasionally a 5 per cent concentration of carbon dioxide was used for a short period before the change to oxygen alone was made

The results will be presented in 2 groups (1) dogs which had interruption of blood flow alone and (2) dogs in which right ventriculotomy was performed during the occlusion period

RESULTS

Twenty one dogs had circulatory arrest for 30 minutes at 17 to 18° C Four of these dogs developed ventricular fibrillation 2 during the occlusion and the other 2 in the post occlusion period 5 and 10 minutes after the release of vascular occlusion These 4 animals died during rewarming although defibrillation had been successful The remaining 17 dogs survived and lived normally for months No evidence of neurological damage could be detected

Right ventriculotomy was performed in 9 additional dogs These were cooled in the same way and the circulation was interrupted at 18° C for periods of 30 minutes except for 2 animals in which the interruption was maintained for only 20 minutes None of these 9 dogs developed ventricular fibrillation and all of them survived and lived for a week or longer No neurological defect could be detected in these dogs

In the majority of these dogs the heart continued to beat regularly during

circulatory arrest but most of them had irregular beats with extrasystoles and idio ventricular or nodal rhythm in the post occlusion period Regular heartbeat with a sinus rhythm returned during rewarming usually at body temperatures between 22° and 25° C

DISCUSSION

The results obtained with this technique, which features the use of carbon dioxide, have not been possible when oxygen alone was used In fact we have not even been able to cool adult dogs to these temperature levels without encountering ventricular fibrillation All of 10 adult dogs given oxygen alone fibrillated at temperature levels between 23° and 19° C during experiments in which the chest was not opened[6]

By using a mixture of carbon dioxide and oxygen with an open, artificial respiratory the blood pH remains relatively constant, at a level slightly below normal, during the entire cooling period[6] This maintenance of a constant blood pH with the avoidance of respiratory alkalosis is probably the most important advantage of the technique If oxygen alone is used with an efficient artificial respirator high blood pH levels occur in the hypothermic animal while if a respirator is not used until later in the cooling period or until the thoracotomy is started a shift in pH from an acid to an alkaline level results Similarly if attempts are made to control the pH by altering the respiratory rate during cooling there is likely to be an alkaline shift in the pH after the chest has been opened This occurs because an increase in respiratory rate or volume is necessary after the chest has been opened in order to avoid collapse of large segments of the lungs With this method the respiratory rate must be reduced gradually as the animal is cooled in order to avoid alkalosis but when the chest is then suddenly opened the volume of respiration at this slow rate is not sufficient to ventilate all of the lung When this is corrected as it must be by increasing the respiratory volume alkalosis occurs It has been well demonstrated that such a shift in pH is apt to cause ventricular fibrillation By using carbon dioxide with an open respiratory system throughout the cooling period and during the thoracotomy these dangerous shifts in pH are avoided

The addition of carbon dioxide has resulted in a startling improvement in our ability to cool animals safely but it alone does not offer an absolute protection against ventricular fibrillation Other factors appear to be important in obtaining the results we have described A careful technique with careful observation of the animal during the entire experiment is essential though this is not enough to protect the animal against fibrillation when oxygen alone is used Still another factor in addition to careful technique may have been important too for these animals required more anesthesia than we have found necessary in dogs cooled with oxygen alone The possible significance of this deep anesthesia has not yet been investigated

These experiments as well as some of our previous work[5, 6] have led us quite understandably to use 5 per cent carbon dioxide in 95 per cent oxygen in our clinical cases undergoing hypothermia for open intracardiac surgery This work has just begun and we have used the technique in only 10 patients undergoing open cardiotomies but the results have been gratifying as there has been no ventricular fibrillation This complication occurred in approximately one third of the patients cooled by our former technique[4]

We hope that this technique will prove to be an important advance capable of lowering the risk and extending the usefulness of clinical hypothermia

SUMMARY

1 The blood flow was arrested in 30 adult dogs for periods of 20 to 30 minutes at body temperatures of 18 C

2 All tolerated this procedure and lived normally for periods of 1 week or longer except 1 which developed ventricular fibrillation The hearts in these dogs were easily defibrillated but the animals died during rewarming

3 Right ventriculotomy was performed in 9 dogs during the circulatory arrest and all of these tolerated the procedure without ventricular fibrillation and survived with no adverse effects

REFERENCES

1 Bigelow W C, Callaghan J C and Hoops J A General hypothermia for experimental intracardiac surgery Ann Surg *132* 531 1950
2 Brown E B and Miller F Ventricular fibrillation following a rapid fall in alveolar carbon dioxide concentration Am J Physiol *169* 56 1952
3 Lewis F J and Taufic M The repair of experimental septal defects during hypothermia with a molded polyvinyl sponge Surg Gyn Obst *100* 593 1955
4 Lewis F J Taufic M Varco R L and Niazi S The surgical anatomy of atrial septal defects experiences with repair under direct vision Ann Surg *142* 401-417 1955
5 Niazi S A and Lewis F J The effect of carbon dioxide on ventricular fibrillation and heart block during hypothermia in rats and dogs In Surgical Forum 1953 Philadelphia W B Saunders Co 1954 p 106
6 Niazi S A and Lewis F J Profound hypothermia in the dog Surg Gyn Obst *102* 98 106 1956
7 Swan H Zeavin M, Holmes J H and Montgomery V Cessation of circulation in general hypothermia I Physiologic changes and their control Ann Surg *138* 360 1953

MYOCARDIAL FAILURE IN EXPERIMENTAL HYPOTHERMIA*

Leo R Radigan Thomas A Lombardo and Andrew G Morrow

The reduction in metabolism which accompanies hypothermia affords substantial protection to the central nervous system during periods of complete interruption of the circulation The myocardium however seems less able to tolerate interruption of its circulation particularly when cardiotomy is performed The high incidence of ventricular fibrillation under these circumstances is recognized Even if the rhythm remains normal experimental animals die in the postoperative period with autopsy findings suggesting myocardial failure The present study was undertaken to investigate this aspect of experimental hypothermia

METHOD

Mongrel dogs weighing 8 to 20 kg were anesthetized with 2 5 per cent sodium pentothal administered intravenously and an endotracheal tube was inserted The dogs were hyperventilated throughout the procedures with 100 per cent oxygen furnished through a demand valve respirator Hypo-

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thermia was induced by immersion in an ice and water bath During cooling small supplemental doses of pentothal were given to prevent shivering

When the rectal temperature was 30° C the animal was removed from the ice bath Using sterile technique a right thoracotomy was performed through the fourth intercostal space and the venae cavae and azygos vein were isolated By this time the rectal temperature had fallen to approximately 28° C The venous inflow was occluded for 8 minutes during which time a 1 cm incision was made in the right ventricle One or 2 sutures were placed and tied in the interventricular septum The ventriculotomy was closed toward the end of the occlusion period The superior cava was released and within 2 minutes the inferior cava The thoracotomy was closed at which time the rectal temperature was usually 25° C

The animals were warmed to 36° C in a water bath maintained at 42° C Right atrial and aortic pressures were recorded before and after inflow occlusion Base line pressure records were made with the transducers leveled at the right atrium Right atrial mean pressure was determined by planimetric integration

Twenty dogs constituted the control group and 20 others were given 0 15 mg of acetyl strophanthidin* intravenously Acetyl strophanthidin exerts a digitalis like effect which is maximal within 5 to 15 minutes and is dissipated within 120 minutes This drug was administered in three doses of 0 05 mg over the 15 minute period

RESULTS AND DISCUSSION

Fourteen animals in the control group (70 per cent) and 7 in the digitalized group (35 per cent) died within 24 hours following the operative procedure Eight of the control animals died after rewarming and 6 died before being rewarmed Autopsies in the former group revealed distension of the venae cavae and fluid in the pleural cavities The lungs were congested Microscopically they showed intense venous engorgement and alveolar edema The hearts were dilated, especially the right ventricles and marked flabbiness of the ventricular musculature was noted The 6 control animals that died during the rewarming period revealed dilated and flabby hearts without associated pulmonary congestion The 6 deaths in the digitalized group occurred after rewarming Autopsy findings in these animals also indicated congestive heart failure

Six of the controls and 13 of the digitalized animals were chronic survivors and exhibited no overt neurologic or cardiovascular abnormalities

The average right atrial mean pressure before inflow occlusion was 3 mm Hg in each group The average mean pressure after inflow occlusion was 7 mm Hg in the control group (range 3 to 11 mm Hg) and 4 2 mm Hg in the digitalized group (range 3 to 9 mm Hg) The pressures in the digitalized group tended to be consistently lower than in the controls Statistical analysis indicated that the differences were significant (p < 01) All of the control animals demonstrated an increase in right atrial mean pressure after release of inflow occlusion The average pressures in the animals that died was 8 2 mm Hg and 5 5 mm Hg in the survivors Four of the 7 digitalized animals that died demonstrated an increase in right

*Kindly supplied by Eli Lilly Co Indianapolis Indiana

atrial mean pressure after inflow occlusion In the remaining [illegible] no rise in pressure was noted

Eighteen of the control dogs (90 per cent) and 9 of the digitalized ([illegible] per cent) developed ventricular fibrillation during the period of inflow occlusion In both groups fibrillation usually occurred when the right ventricle was incised or when a suture was placed into the interventricular septum All the hearts were defibrillated with massage and electric shock Five of the 6 survivors in the control group and [illegible] of [illegible] survivors in the digitalized group fibrillated during the period of inflow occlusion

It was considered that the foregoing experiments demonstrated hemodynamic and pathologic evidence of myocardial failure This was presumed to be the result of inadequate coronary circulation In order to estimate the changes in the coronary outflow during the period of circulatory occlusion another series of animals was studied

After cooling a right thoracotomy was performed and the right atrium was opened during a period of inflow occlusion Ventricular fibrillation was prevented by prior infiltration of the superior atriocaval junction with 1 per cent procaine[3] A polyethylene catheter was placed into the orifice of the coronary sinus and held in place with a pursestring suture The atrial wall was closed about the catheter which was led to a graduated cylinder and normal circulation established This catheterization was accomplished with approximately 2 minutes of inflow occlusion The end of the catheter was leveled to prevent siphoning and determinations of the flow and oxygen

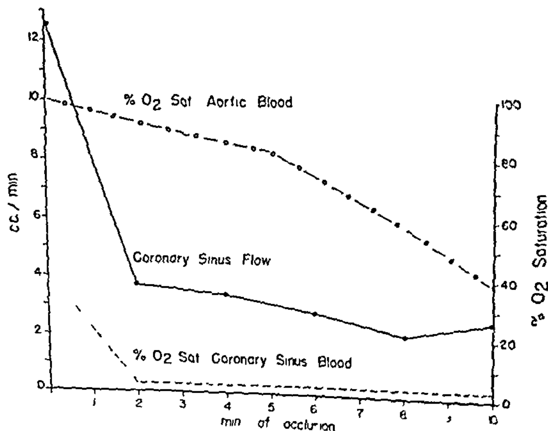


Fig 1 Coronary sinus flow and oxygen saturation of coronary sinus and aortic blood before and during inflow occlusion

content of the coronary sinus blood made with and without circulatory occlusion Simultaneously, samples of blood were taken periodically from the ascending aorta and analyzed for oxygen content Fifteen animals were studied utilizing the previously described standard operation but with 10 minutes of circulatory occlusion The results are depicted in Figure 1

Prior to occlusion the coronary sinus flow averaged 12 5 cc /min Two minutes after inflow occlusion coronary sinus flow decreased to 3 5 cc /min and was maintained near this level throughout the remainder of the period of occlusion The oxygen saturation of the coronary sinus blood approached zero after 2 minutes of occlusion Throughout the period of occlusion there was progressive desaturation of aortic blood and at the end of 10 minutes the average saturation was 10 per cent This was attributed to the admixture of venous blood from channels draining into the left ventricular cavity This observation was unaltered by simultaneous pulmonary venous occlusion in 3 additional dogs

The normal right atrial mean pressure and normal oxygen content of coronary sinus blood before inflow occlusion suggest little if any impairment of myocardial function It was therefore assumed that coronary circulation was adequate at a temperature of 28° C The total coronary flow at this temperature was estimated to be approximately twice the observed coronary sinus flow or about 25 cc /min in animals weighing 10 to 12 kg Eight animals of this weight were subjected to the standard operative procedure and the coronary arteries were perfused with arterial blood collected from a donor animal The blood was delivered by gravity from a transfusion bottle into the isolated proximal aorta The period of inflow occlusion varied from 16 to 20 minutes Two animals were perfused with 12 and 16 cc /min They died in obvious heart failure following release of the circulation The remaining 6 were perfused with 25 to 35 cc /min and survived without sequelae Two of these animals demonstrated slight elevations of right atrial mean pressure after release of the circulation The pressure returned to normal following the administration of acetyl strophanthidin (0 15 mg) to these dogs

SUMMARY

Hypothermic animals subjected to inflow occlusion and right ventriculotomy demonstrated elevations in right atrial mean pressure and pulmonary congestion These evidences of myocardial failure were partially reversed by a rapid acting digitalis preparation acetyl strophanthidin In addition digitalization increased survival and decreased the incidence of ventricular fibrillation

The myocardial failure was attributed to inadequate myocardial oxygenation brought about by diminished coronary flow and progressive desaturation of blood supplied to the coronary arteries during inflow occlusion Hypothermic animals survived cardiotomy and inflow occlusion of 20 minutes when the coronary system was perfused with small volumes of oxygenated blood

REFERENCES

1 Shumacker H B Jr Cardiovascular surgery Henry Ford Hospital Symposium Philadelphia W B Saunders Co 1955

THE EFFECT OF PROLONGED HYPOTHERMIA ON OXYGEN CONSUMPTION OF THE LIVER SLICE*

EDWARD J. FEDOR, MORTON LEVINE, CLEM RUSS AND BERNARD FISHER

Swan[1], in a review of the present status of hypothermia, suggested that this modality might be employed in the therapy of such conditions as acute pulmonary disease, anemic crises, shock and myocardial infarction. If this be so, then it is conceivable that prolonged hypothermia would be utilized. It has been reported that total body oxygen consumption in acute hypothermia is diminished by decreasing the body temperature.[2] However, no effect on the rate of O_2 consumption of liver, heart and brain in the dog[3] and of the liver slice in the rat[4] has been found. What effect prolonged hypothermia and rewarming of the animal would have upon the metabolic activity of these tissues has not been reported. Following protracted cold and subsequent warming, the oxygen uptake of the liver slice, as an index of tissue alteration, and the determination of composition of the liver has been made. This data represents the basis of this report.

METHOD

Mongrel adult dogs fasted 16 hours were injected with 1 cc. sodium pentobarbital and 1/150 gr. atropine sulfate. Following this sedation the animals were anesthetized by open drop ether and immersed in a cold water bath at 1° C. until the rectal temperature read 28° to 29° C. At this time the animals were placed in an air conditioned room and body temperature was maintained at 22° to 24° C. by regulating the environmental temperature. With ether as the anesthetic agent it has been found[5] that at 22° to 24° C. respirations were maintained without mechanical assistance. Rewarming was instituted by placing the dogs in a warm bath (40° C.) until the body temperature had risen to 28° or 29° C. They were then dried with towels and placed in a warm room where they were permitted to return to precooling temperatures.

All samples of liver were treated similarly. After excision of a liver lobe duplicate samples weighing 0.5 to 1.0 gm. were introduced into centrifuge tubes containing KOH. Glycogen determinations on these samples were determined by the method of Good, Kramer and Somogyi.[6] Oxygen consumption was measured at 37° C. in a manner described by Umbreit, Burris and Stauffer.[7] Uniform liver slices obtained by using a Stadie Riggs microtome were suspended in an oxygenated Krebs Ringer phosphate solution with and without substrate (0.018/M glucose). Oxygen uptake is expressed as μl of oxygen consumed per mg. of dry weight tissue over a period of 4 hours. The percentage of water was determined by drying samples to a constant weight in an oven at 100° C. Total fatty acids were ascertained by alcoholic potassium hydroxide saponification and petroleum ether extraction of liver homogenate. Total nitrogen and protein nitrogen determinations were made on samples using the Hengar method according to Wagner.[8] Blood glucose was determined by the method of Somogyi as

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modified by Nelson[9] Values of water, protein glycogen and fatty acids are presented as grams per 100 gm of wet liver (percentage)

RESULTS

Data presented in Table 1 demonstrates average values for oxygen uptake of the liver slice and hepatic glycogen in 12 normothermic dogs which were anesthetized by open drop ether and served as controls Fifteen animals cooled from 1 to 5 hours, did not differ significantly in the Q_O from uncooled dogs but the decline of liver glycogen was significant Subsequent to 6 hours of cooling a sharp decrease in oxygen consumption was observed, although in the 6 to 10 hour animals the glycogen level was unchanged from the 1 to 5 hour group Glycogen content continued to fall in the 12 to 28 hour hypothermic dogs, but the oxygen uptake remained unchanged from the 6 to 10 hour group By these data it can be assumed that in the dog the hepatic glycogen level is not directly related to oxygen consumption of liver slices contrary to the findings of Fuhrman and Crismon[4] Whether some other component of the liver might be a factor in oxygen uptake prompted us to include determination of the nitrogen fatty acids and water fractions of the liver In Table 2 the mean amount of water protein and fatty acids in the liver at various hours of hypothermia are presented There were no significant changes in water and total nitrogen however there were significant decreases in the protein and protein nitrogen and the 1 to 5 hour fatty acid fractions In the 6 to 10 hour group fatty acids were restored This change in the fatty acid component may be explained by fat stores (*element variable*), which are extremely mobile and does not

Table 1 Average Oxygen Uptake and Glycogen Content of Liver before and after Hypothermia

NO OF DOGS	HOURS OF HYPOTHERMIA	Q_{O_2} WITHOUT GLUCOSE	Q_{O_2} WITH GLUCOSE	GLYCOGEN %
12	0	10.6 (1.1)	10.6 (1.3)	3.5 (0.3)
15	1 5	11.2 (1.8)	11.6 (2.4)	1.4 (1.3)
8	6 10	5.4 (1.6)	5.9 (1.7)	1.5 (0.9)
10	12 28	5.1 (1.3)	5.9 (2.0)	0.4 (0.2)

( ) figures in brackets represent standard deviation of the mean

Table 2 Average Composition of Liver before and after Hypothermia

NO OF DOGS	HOURS OF HYPOTHERMIA	TOTAL N %	N P N %	PROTEIN N %	PROTEIN %	FATTY ACIDS %	WATER %
12	0	28.2 (2.1)	4.8 (0.3)	23.4 (2.0)	14.8 (1.1)	4.6 (1.0)	72.6 (2.0)
7	1 5	26.6 (1.4)	7.3 (1.9)	19.3 (1.1)	12.1 (0.6)	3.2 (1.1)	74.4 (1.4)
10	6 11	28.9 (2.3)	7.8 (1.8)	21.1 (2.2)	13.2 (1.3)	4.2 (0.8)	73.8 (1.5)
6	12 28	29.6 (4.2)	9.5 (1.5)	20.2 (2.3)	13.3 (0.7)	4.8 (0.6)	72.8 (1.2)

( ) figures in brackets represent standard deviation of the mean

Table 5 Oxygen Uptake and Composition of Liver following Rewarming of Hypothermic Dogs

DOG NO	HOURS OF HYPOTHERMIA	HOURS OF REWARMING	QO_2 WITHOUT GLUCOSE	QO_2 WITH GLUCOSE	GLYCOGEN %	T N	N P N %	P N %	PROTEIN %	FATTY ACIDS	WATER %
43A	6	5	11 9	10 1	2 0	29 6	7 6	22 0	13 8	[illegible]	71 [illegible]
92A	6	6	12 9	11 7	1 9	30 [illegible]	3 4	27 1	17 1	1 6	73 4
81A	6	6	12 0	11 7	0 4	29 6	1 0	2[illegible] 6	16 0	1 6	71 0
69A	6	7	8 1	9 7	4 6	20 4	3 2	[illegible]6	12 8	–	7[illegible]
8A	6	7	9 3	7 7	0.5	27 2	[illegible]6	21 6	13 [illegible]	1 9	7[illegible] 7
Mean			10 8 (1 8)	10.2 (1.5)	1 9 (1 5)	27.[illegible] (3 7)	4 7 (1 6)	[illegible]0 (2.2)	14 6 (1 [illegible])	1 9 (0 [illegible])	7[illegible] (1 7)
89A	12	4	10 9	11.2	0 4	31 7	1 3	[illegible]7 4	16 8	1 7	7[illegible]
201A	12	6	23 8	26 4	0.[illegible]	30.2	7 9	[illegible]3	13 9	[illegible]0	7[illegible] 3
244A	12	8	19 0	18 7	1.2	30 9	[illegible]0	2[illegible] 9	16.2	[illegible]	[illegible] 0
693	12	6	10 3	9 7	0 1	28 8	4 2	21 6	1[illegible] 4	[illegible] 1	[illegible]
253A	12	6	17 4	18 4	0 1	29.2	3 9	[illegible]3	1[illegible] 8	[illegible]	7[illegible]
Mean			16 3 (5 1)	16 9 (6 0)	0.5 (0 1)	30.2 (1 1)	[illegible] (1 [illegible])	[illegible]1 (1 7)	1[illegible] 6 (1 0)	[illegible] (0 [illegible])	7[illegible] (1[illegible])

( ) figures in brackets represent standard deviation of the mean

Table 4 Oxygen Uptake and Glycogen Content of Liver Slice and Blood Glucose in Hypothermic Dogs

DOG NO	HOURS OF HYPOTHERMIA	QO_2 WITHOUT GLUCOSE	QO_2 WITH GLUCOSE	BLOOD GLUCOSE MGM %	GLYCOGEN %
136A	1	9.5	9.5	79.1	4.4
978	2	9.1	8.7	147.7*	3.1
145A	3	10.4	10.5	111.3	2.1
180A	4	12.4	12.3	141.0*	0.4
34A	7	4.1	4.5	305.9†	1.1
13A	9	4.6	4.0	109.6	1.8
18A	10	6.5	8.0	83.7	2.9
959	14	6.3	9.8	281.7†	0.6
903	20	6.2	5.7	76.4	
979	24	3.0	3.7	34.0	1.0
850	28	3.6	4.1	200.0†	

*Shivering
†5% glucose I V

necessarily indicate a change in structural lipids The significant decrease of protein and protein nitrogen and the significant increase in non protein nitrogen observed in Table 2 could be attributed to a demand of the body to maintain a normal blood sugar level by conversion of amino acids to sugar, thereby lowering liver protein and protein nitrogen The elevation of liver non protein nitrogen might be due to an inability of liver cells to excrete non protein nitrogen products into the blood stream However if the changes in the nitrogen portion of the liver had any direct effect on oxygen uptake this should have been revealed in animals cooled from 1 to 5 hours for even in this group the protein and protein nitrogen have significantly decreased and the non protein nitrogen significantly increased That these changes in liver composition during hypothermia are of a transient nature can be observed in Table 3 which indicates a series of animals subjected to either 6 or 12 hours of hypothermia and then allowed to rewarm to the pre cooling level In the 6 hour hypothermic group we noted a return to normal for all factors except liver glycogen The 12 hour group after rewarming exhibited the same trend and in addition the Q_O for 3 dogs significantly above the control level Here again there seems to be no relationship between the hepatic glycogen level and oxygen uptake of the liver What effect if any does blood glucose have on oxygen consumption is indicated in Table 4 In the 3 animals (34A 959 and 850) where a 5 per cent glucose solution was administered the Q_O was not maintained at normal values This would indicate that blood glucose *per se,* is not a direct factor in the maintenance of liver oxygen consumption

DISCUSSION

Following hypothermia of more than 6 hours duration there is a significant decrease in oxygen consumption in the liver slice of the dog Prior to 6 hours the Q_O is maintained at a normal level This is similar to the findings of Burdette[3] and Fuhrman[4] in acute hypothermia However changes

in liver composition do occur in the period when the oxygen uptake does not change significantly. This would seem to indicate that in these experiments no quantitative relationship exists between liver glycogen, fatty acids and protein and oxygen consumption by liver slices. This alteration in liver composition probably involves the labile protein and fatty acids because following rewarming liver composition is once again normal. Therefore the decrease in oxygen uptake might be explained as being due to a depressing effect of prolonged subnormal temperature on an enzyme system or systems. However, if prolonged coldness alone had such an effect certainly it might be expected that by the end of the third hour in a Krebs Ringer phosphate solution at 37° C, this would be overcome. Liver slices respire relatively the same over the entire 4 hours period. It is probable that the enzyme system or systems are somehow altered during prolonged hypothermia and that during the *in vivo* rewarming process these enzymes are restored to a normal state eventuating in normal oxygen consumption. The addition of a substrate (0.018/M glucose) to manometric flasks did not alter the Q_O significantly. Maintaining a high glucose level in the blood of prolonged hypothermic animals did not maintain a normal Q_O. However other substrates might have a normalizing effect on oxygen consumption in liver slices and should be investigated.

In conclusion it must be stated that these data substantiate previous findings[5] that prolonged hypothermia is not similar to hibernation since changes in Q_O become greater with the length of hypothermia although the temperature may remain stable.

CONCLUSIONS

1. Oxygen uptake of liver slices was not altered by 1 to 5 hours of hypothermia.

2. From 6 to 28 hours of cooling the respiration of liver slices was significantly decreased.

3. Significant alterations in liver glycogen, protein nitrogen, protein and non protein nitrogen occurred. The 1 to 5 hour hypothermic group demonstrated a significant decrease in fatty acids.

4. Rewarming animals to pre cooling levels restored the Q_O to normal or above normal and liver composition to normal.

5. Oxygen consumption of liver slices in the dog was not affected by glycogen content of liver and blood glucose.

REFERENCES

1. Swan, H. The current status of hypothermia. A.M.A. Arch. Surg. *69*:597, 1954.
2. Bigelow, W. G., Lindsey, W. K., Harrison, R. C., Gordon, R. A. and Greenwood, W. F. Oxygen transport and utilization in dogs at low temperature. Am. J. Physiol. *160*:125, 1950.
3. Burdette, W. J. Oxygen consumption of tissues after anesthesia at low temperature. In Surgical Forum 1952. Philadelphia, W. B. Saunders Co., 1953, p. 655.
4. Fuhrman, F. A. and Crismon, J. A. The influence of acute hypothermia on the rate of oxygen consumption and glycogen content of the liver and on the blood glucose. Am. J. Physiol. *149*:552, 1947.
5. Fisher, B., Russ, C., Fedor, F. J., Wilde, R., Engstrom, P. and Prendergast, P. Experimental evaluation of prolonged hypothermia. Arch. Surg. (In press).
6. Good, C. A., Kramer, H. and Somogyi, M. The determination of glycogen. J. Biol. Chem. *100*:485, 1933.

7 Umbreit W W Burris R H and Stauffer J F Manometric techniques and tissue metabolism Minneapolis Minn Burgess Publishing Co 1951
8 Wagner E C Titration of ammonia in presence of boric acid Indust Engin Chem 12 771 1940
9 Nelson N A photometric adaptation of the Somogyi method for determination of glucose J Biol Chem 153 375 1944

INHIBITION OF THE STRESS RESPONSE DURING SURGERY UNDER HYPOTHERMIA*

WILLIAM F BERNHARD AND JAMES D MCMURREY

Although the clinical application of hypothermia in the care of surgical patients has become increasingly important the metabolic response of the organism to operative stress in hypothermia has not as yet been clearly defined Recently direct methods for measurement of 17 hydroxycorticoids in the blood were made available and determinations have been carried out in normal individuals and in patients during and after surgery at normal body temperature[1, 2] This study concerns the determination of 17 hydroxy corticoids in the adrenal venous blood of hypothermic dogs and the peripheral level of 17 hydroxycorticoids in the dog and man before during and after surgery under hypothermia

The Output of 17 Hydroxycorticoids from the Adrenal Vein in the Hypothermic Dog A group of mongrel dogs were anesthetized with pentobarbital sodium and maintained with small amounts of ether utilizing a positive pressure apparatus The right adrenal gland was exposed and the lumbo adrenal vein isolated and cannulated A plastic cord was passed around the vein between the adrenal gland and the entrance of the lumbo-adrenal vein into the vena cava Tension on this cord (snare) produced a retrograde flow of all adrenal venous blood and permitted sampling Upon the release of the snare the adrenal blood flowed normally into the inferior vena cava

Adrenal venous blood was collected directly into heparinized graduated test tubes and the blood flow per minute recorded The plasma 17 hydroxy corticoids were determined by the method of Silber and Porter[3] and the output was calculated in gammas per minute assuming a hematocrit of 50 per cent The first sample was obtained immediately after the right flank wound had been closed and represented the adrenal cortical output from a maximally stimulated adrenal gland ($8\ 6 \pm 0\ 7$ gamma per minute) † The response to ACTH was then tested by the intravenous administration of 10 milliunits of USP reference standard ACTH dissolved in 10 cc of 0 01 normal hydrochloric acid in saline solution The adrenal cortical output was not increased however and confirmed the impression that all values obtained in this experiment were from a maximally stimulated gland

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*From the Surgical Service Peter Bent Brigham Hospital and the Laboratory for Surgical Research Harvard Medical School This work was sponsored by the Committee on Metabolism in Trauma Commission on Liver Diseases Armed Forces Epidemiological Board and supported in part by the Surgeon General Department of the Army through a contract (DA-49 007 MD-472) with Harvard University The generous assistance of the Samuel Cabot Fund is gratefully acknowledged

†Standard Error of the mean

One hour after induction of hypothermia a standard operative stress was performed on each animal and further samples obtained. Cooling of the animals to 30° C produced a small drop in adrenal blood flow and hence a fall in corticoid output (5.0 ± 0.8 gamma per minute). At a mean temperature of 21° C adrenal blood flow had decreased markedly without a significant rise in corticoid concentration, resulting in a fall in adrenal corticoid output (2.0 ± 0.1 gamma per minute). Additional surgical stress during hypothermia did not increase the mean corticoid output (2.0 ± 0.1 gamma per minute) nor did this output change appreciably after further ACTH stimulation (2.2 ± 0.2 gamma per minute). When the animals had rewarmed to 30° C, adrenal flow and concentration increased simultaneously and at 37° C the corticoid output again reached the pre-cooling level.

In a group of 5 animals hemorrhagic shock was produced prior to the induction of hypothermia. Blood was removed rapidly (15 minutes) from a femoral artery and collected in a plastic bag to permit prompt replacement. This was done to rule out the fall in blood pressure associated with hypothermia as the cause of the adrenocortical inhibition. After stabilization of the mean blood pressure at 15 mm Hg, adrenal venous samples were taken and retransfusion carried out immediately. The data from these animals indicated that at normal temperature there was an associated marked rise in the concentration of 17-hydroxycorticoids with a concomitant fall in adrenal blood flow to the same level observed during hypothermia. Because of this response, the total hormone output remained at or near pre-shock levels. This confirmed the work of Hume and Nelson who found a similar rise in concentration associated with a fall in flow during shock.[4]

These studies indicated that there was a decrease in adrenal blood flow with hypothermia and a fall in 17-hydroxycorticoid output. This low output was not altered by additional surgical stress or the injection of ACTH. With rewarming of the animals, normal adrenal blood flow and 17-hydroxycorticoid output returned promptly to the pre-cooling level. Hemorrhagic shock in a small group of animals at normal body temperature produced a marked reduction in adrenal blood flow but an associated rise in corticoid concentration in the adrenal venous blood. In this case total corticoid output did not fall. Therefore it was concluded that a specific depression of adrenocortical hormone secretion occurred in these animals during hypothermia.

Peripheral Serum Levels of Free 17-Hydroxycorticoids in the Dog under Hypothermia. Having determined the inhibition of the adrenal cortical 17-hydroxycorticoid output at low temperature in adrenal venous blood, the peripheral corticoid level was then investigated. This level is determined not only by adrenal cortical production of 17-hydroxycorticoids but also by their rate of utilization, conjugation, and excretion. Concomitant depression of these 3 processes may be anticipated in hypothermia since cooling depresses tissue metabolism throughout the organism.

A second group of adult mongrel dogs was anesthetized and prepared as previously described. In these animals a polyvinyl cannula was inserted into a femoral artery permitting arterial blood sampling. An initial laparotomy was then carried out and closed, following which the first peripheral blood sample was obtained. This represented the control level from a surgically stressed normal animal. Hypothermia was then induced

and after a period of stabilization a second laparotomy was performed and another arterial blood sample collected In all animals the inferior mesenteric superior mesenteric, celiac axis, hepatic artery and portal vein were isolated and occluded with bull dog clamps, permitting complete exclusion of the liver from the circulation[5] The occlusions were maintained for 1 hour, and then the third arterial blood sample was obtained The clamps were then released, the incision closed and the animals rewarmed in a warm water bath The final arterial blood sample was obtained when the body temperature returned to normal

The mean 17 hydroxycorticoid levels in the arterial plasma of the dogs studied revealed a plasma corticoid level of 17 8 ± 1 5 gamma per cent after the control laparotomy at normal body temperature After the induction of hypothermia and stabilization of the animal s temperature at 25° C a second laparotomy was performed The mean corticoid concentration at the end of this procedure was 21 8 ± 3 5 gamma per cent After 1 hour of exclusion of the liver from the circulation the level was 23 7 ± 3 6 gamma per cent Finally after a warming to normal body temperature the level was 25 0 ± 2 8 per cent

The half life of hydrocortisone in the blood of the normal animal is less than 1 hour the hormone being conjugated by the liver excreted in the urine and possibly utilized by the tissues The fact that the arterial corticoid levels in the animals studied increased rather than decreased after 2 hours of hypothermia and further surgical trauma suggests that depression of these mechanisms is equal to or greater than the fall in adrenocortical production of the hormones This conclusion was strengthened by the fact that exclusion of the liver from the general circulation failed to produce a change in the arterial corticoid levels If the liver were conjugating steroids at a normal rate in the hypothermic animal an appreciable fall in plasma corticoid level would have resulted It is evident, therefore that the marked reduction in adrenocortical hormone production in hypothermia is more than balanced by the concomitant depression of mechanisms which normally tend to reduce this level

Peripheral 17 Hydroxycorticoids in Surgical Patients A small group of patients undergoing surgery with hypothermia have also been studied Peripheral venous blood samples were obtained before during and for 1 to 2 weeks following surgery Blood was collected in dry test tubes allowed to clot and the serum removed Samples were stored in a deep freeze until analyzed, at which time the corticoid determinations were carried out by the method of Nelson and Samuels[1] The normal range for peripheral *17 hydroxycorticoids in this laboratory is 5 to 15 gamma per cent* with a mean of 10 gamma per cent Additional patients undergoing surgery under general anesthesia at normal body temperature have been included in this study for comparison with the hypothermia cases

In the patients studied the peripheral corticoids also revealed a constant level during the course of surgery under hypothermia These steroid values are different from those obtained in the experimental animal in that the patients were not subjected to surgical stress prior to cooling The stress response provoked by anesthesia *per se* was not marked and with cooling plus extensive surgery there was no significant rise in corticoid levels Furthermore the values remained within the normal range for several

hours after completion of rewarming A postsurgical rise did occur as revealed by samples obtained within the next 12 hours However in all cases the magnitude of the response was less than that expected from the degree of surgical trauma that took place

The usual pattern of 17 hydroxycorticoids during anesthesia and surgery at normal body temperature is characterized by an initial steep rise with induction of anesthesia reaching levels 4 to 6 times the resting value[2] Peak levels are generally reached within 2 to 3 hours following the conclusion of the operation Thereafter a sharp fall occurs with normal values again present 14 to 72 hours later This type of response has been recorded in numerous patients undergoing abdominal surgery with pentothal ether anesthesia

SUMMARY

1 A group of animals were prepared by cannulation of the right adrenal vein and hypothermia induced by surface cooling Adrenal blood flow and 17 hydroxycorticoid concentration were determined before during and after cooling The responses to ACTH and surgery were determined before and during cooling Hemorrhagic shock was induced in a small group of animals and adrenal venous samples collected to compare the effects of shock with those of hypothermia

2 Hypothermia was associated with a decrease in adrenal blood flow and a resultant fall in 17 hydroxycorticoid output This was not altered by additional surgical stress or the injection of ACTH Normal adrenal blood flow and 17 hydroxycorticoid output returned promptly with rewarming

3 Hemorrhagic shock in animals at normal body temperature produced a marked reduction in adrenal blood flow but an associated rise in corticoid concentration in the adrenal venous blood Therefore total corticoid output did not fall

4 In a second group of animals the peripheral arterial level of plasma 17 hydroxycorticoids were determined after laparotomy at normal body temperature and during hypothermia The effect of exclusion of the liver from the circulation was evaluated

5 The peripheral venous corticoid levels were followed in a small group of patients subjected to surgery under hypothermia and compared with the response to surgery in normothermic patients The dogs and patients revealed constant peripheral corticoid levels with extensive surgical trauma during hypothermia

6 Hypothermia with a concomitant reduction in body metabolism simultaneously depressed production and conjugation of the steroid hormones and to a similar degree

7 A post surgical rise in peripheral corticoids does occur but the magnitude is less than that expected with comparable major surgery performed at normal temperature

8 Immersion cooling *per se* did not provoke a stress response as measured by peripheral 17 hydroxycorticoids

REFERENCES

1 Nelson D H and Samuels L T A method for the determination of 17 hydroxycorticosteroids in blood J Clin Endocr Metab *12* 519 1952

2 Steenburg R W Lennihan R and Moore F D Studies in surgical endocrinology II The free blood 17 hydroxycorticoids in surgical patients their relation to urine steroids metabolism and convalescence Ann Surg (In press)
3 Silber R H and Porter C C The determination of 17 21 dehydro 20 ketosteroids in urine and plasma J Biol Chem *210* 923 1954
4 Hume D M and Nelson D H Adrenal cortical function in surgical shock in Surgical Forum 1954 Philadelphia W B Saunders Co 1955 p 568
5 Bernhard W F McMurrey J D and Curtis G W Feasibility of partial hepatic resection under hypothermia N England J M *253* 159 1955

RESERVOIR CIRCULATION WITHOUT MECHANICAL PUMPS FOR INTRACARDIAC SURGERY UNDER DIRECT VISION*

JOHN C CALLAGHAN, FRANK GERBODE MARTIN BIRNSTINGL AND BENJAMIN BELMONTE

It has been well established that survival of living organisms is possible under conditions of delivery of oxygen to the tissues in amounts much less than those used by the tissues under calculated basal conditions Wiggers[1] after a prolonged study of post hemorrhagic hypotension found that at least 15 minutes of systemic blood pressures of 50 mm of Hg or less must be maintained before evidences of low nutrient flow begin to appear in dogs Isolated complete recovery from prolonged periods of cardiac arrest during which manual massage of the heart was the only *vis a tergo* gives support to the applicability of this thesis to humans [3, 4]

Andreason[5] working in England reported the results of experiments in dogs in which he was able to obtain survivals from prolonged venous inflow occlusion of the heart provided that the flow returning via the azygos vein was unimpeded This amount of blood returning to the heart when added to the outflow from the coronary sinus and thebesian veins provided the minimal cardiac output from the left ventricle which was compatible with life under these conditions Lillehei applied this concept in dogs[6] and later in human cases to perform intracardiac surgical procedures under direct vision A donor human circulation was used in those clinical cases to provide the source of oxygenated blood and mechanical pumps were used to balance inflow and outflow between the two circulations

Human survival from periods of such reduction in flow immediately brings the problem of cardiac substitution at least in children to the delivery of relatively small amounts of oxygenated blood For brief periods of cardiac exclusion the amount of blood required is well within the range of that which could be collected in advance as a reservoir of oxygenated *blood calculated to provide the amount of blood needed for the period of* cardiotomy The elimination of the donor human circulation is therefore possible and desirable[7, 8]

This report contains the results of experiments on 10 consecutive dogs subjected to bypass of the heart and lungs with maintenance of the systemic circulation by delivery of oxygenated blood from a reservoir by manual

*Stanford University School of Medicine San Francisco California Aided by a grant from the Life Insurance Medical Research Fund

expulsion from plastic transfusion bags Ventriculotomy was performed in each experiment

METHOD

Preparation of the Reservoir of Oxygenated Blood Two sources of arterial blood were used The first was that of total withdrawal of arterial blood from the femoral artery of sacrificed dogs Large dogs between 10 and 30 kg were used These animals were anesthetized with intravenous nembutal in doses of 30 mg per kg of body weight and then intubated The femoral artery was cannulated with a polyethylene catheter of 2 mm internal diameter and the blood passed through as short a length of polyethylene as possible into especially constructed siliconed plastic transfusion bags* Prior to the entry of the blood each bag had been injected with a solution containing 25 cc of 5 per cent dextrose in saline as a platelet nutrient and 20 mg of heparin This was found to be sufficient anticoagulant to prevent 600 cc of blood from clotting regularly The filled bag after all traces of air or oxygen bubbles were expelled from the outlet was then ready for administration

The second source of blood was that of venous blood drawn as above but from the femoral vein directly into the plastic bags Three hundred cc were drawn into each bag prepared as for arterial blood and 200 cc. of oxygen inserted Gentle agitation by a rocking movement provided a gradual change of color of the blood as the saturation rose to values over

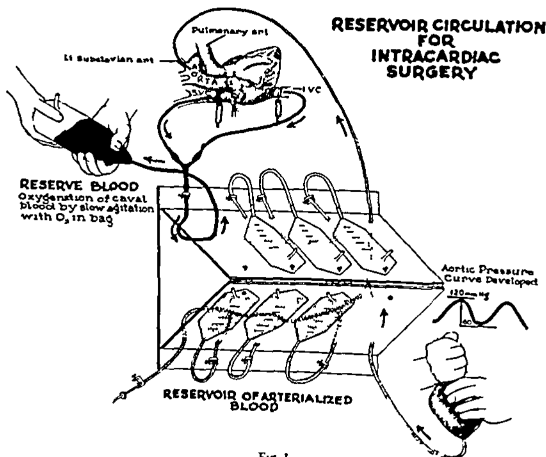


Fig 1

*Made by Cutter Laboratories Berkeley California

90 per cent within the 10 minute period of agitation. The minimal froth that occurred was expressed from the outlet of the bag along with the surplus oxygen and the bag was then ready for use in the arterial reservoir. A study was made of the platelet counts, oxygen saturation, fibrinogen content, pH and carbon dioxide content at the various stages of the oxygenation procedure and will be the basis of a separate report.

Delivery of Arterial Blood to the Animal. Pulsatile flow was obtained by firmly compressing the plastic bag rhythmically at approximately 10 per minute rate, observing the giant pulse obtained in the systemic vessels of the animal on either a cathode ray oscilloscope or a simple mercury manometer indicating the pressure curve in the lower aorta or iliac vessels. Careful timing of the delivery was estimated ahead of time so that the time for completion of each bag was known and control of rate or volume of flow per squeeze could make up deficit or decrease surplus at each time interval. In the dogs of the size used in this series each bag lasted between 2 and 3 minutes.

Table 1. Results of Right Ventriculotomy on 10 Consecutive Dogs using Reservoir Circulation

DOG NO	WEIGHT	DURATION OF CLAMP OFF	FLOW RATE	RESULT	FOLLOW UP
B 16	8.7 kg	6 min	20 cc/kg/min	Survival	Alive 3 mos
B 17	7.8	6 min	25 cc/kg/min	Survival	Alive 3 mos
B 18	7.6	6 min	30 cc/kg/min	Survival	died 4 weeks peritonitis
B 19	8.9	6 min	23 cc/kg/min	Survival	Alive 3 mos
B 20	8.9	9 min	23 cc/kg/min	Survival	Alive 3 mos
B 21	9.8	9 min	30 cc/kg/min	Survival	Alive 3 mos
B 22	9.6	8 min	30 cc/kg/min	Survival	Alive 3 mos
B 23	7.5	8 min	30 cc/kg/min	Survival	Alive 3 mos
B 24	5.9	6 min	no flow 3 min	Died	Technical error
B 26	10.0	8 min	30 cc/kg/min	Survival	Alive 3 mos

The 1 technical death in dog Number B 24 resulted from the failure to check the retaining clamps on the other bags with the result that there was a period without any flow until the error was recognized. In spite of the increased flow for the remaining period of the occlusion which was such that the average flow was normal, the animal entered ventricular fibrillation. After resuscitation by a single electric shock he died 4 hours later having failed to regain consciousness. The final dog in the series had a transient ventricular fibrillation following release of the caval occlusion. This was immediately corrected by a single shock and the animal recovered uneventfully.

In each of the animals a high volume pulse was created with systolic peaks between 100 to 150 mm Hg and the diastolic pressures falling to 10 to 50 mm Hg before commencing the next beat in order to create a large pulse pressure which has been felt to be important under these conditions of low flow to the tissues.

The absence of delayed death in the animals surviving the procedure is felt to be indicative of adequate cerebral flow during the cardiac occlusion

On weighing the animals before and after the procedure weight change was found to vary between minus 50 gm to plus 200 gm

With dogs Number B 20 21 22 23 26 where the clamp-off was longer than 6 minutes their own caval blood was returned on the arterial side after a period of oxygenation of 5 minutes

Surgical Procedure The dogs used for the surgical portion of the study varied in weight between 5 9 and 12 0 kg were mongrels and unselected Each received ¼ grain of morphine subcutaneously 1 hour before induction of anesthesia by intravenous use of nembutal in doses of 20 mg per kg After shaving and intubation the animals were carefully weighed so that this weight could be compared with the immediate postoperative weight of the animal as a check of satisfactory balance of blood volume A polyethylene tube of 0 5 mm internal diameter was connected via a Statham transducer to a multichannel electronic recorder Continuous electrocardiographic visualization was observed along with the pulse tracing throughout the procedure Caval catheters of polyethylene were either inserted separately through femoral and external jugular veins and passed along them until they lay in the superior and inferior vena caval veins respectively or together via the right atrial appendage Each caval catheter had an internal diameter of 2 mm The arterial catheter also inserted via the left subclavian artery and passed down into the arch of the aorta A firm vinyl catheter was used and inserted via a separate stab incision in the anterior chest wall so that its line was directly that of the artery into which it was to be placed The internal diameter of the arterial catheter was 2 mm or 3 mm Just prior to the insertion of the cannulae the animals received heparin in doses of 1 mg per kg body weight

The cavae were clamped as the arterial inflow was started Caval return was then started 10 seconds later The pulmonary artery was obstructed by a bulldog clamp The right ventricle was then opened widely and the bright red arterial bleeding from its cut margins was noted as was the amount of blood entering the ventricle from the coronary sinus and thebesian veins The clamp-off varied between 6 and 10 minutes The ventricle was then closed with continuous suture of 0000 ethicon the clamp on the pulmonary artery removed and the venae cavae released Further arterial inflow or venous takeoff was carried out depending on the protamine titration resul[illegible] The clotting times in each case at the time the animals were returned [illegible] their cages varied between 3 to 12 minutes The animals were then weig[illegible] carefully as a check on blood volume balance A slow transfusion of [illegible] blood was given during the early postoperative period

RESULTS

Of the 10 consecutive animals undergoing the procedure 9 surviv[illegible] of the survivals died at the end of the fourth week of periton[illegible] perforated bowel after 2 weeks of normal postoperative progress [illegible] maining 8 animals have survived 3 months and are apparently [illegible]

The 9 surviving animals displayed no evidence of bleedin[illegible] the chest or into the incisions After a 2 day period of [illegible] became frisky and active and [illegible] active

DISCUSSION

The simplicity of the procedure is reflected we believe in the high percentage (90 per cent) of survivals from the ventriculotomy in the series. The technical error that resulted in the 1 death of the series was due to a failure to carry out a careful check so necessary in any form of extracorporeal system.

During the early pilot experiments prior to starting the series here reported we were impressed with the increased survival rate and improvement in the condition of the animals when a pulse was present in the system as compared to a mean flow setup as is present in most of the pumps systems used. Possibly there is an economy exercised by a pulsatile flow to the tissues when the flows are minimal. In addition, the large volume pulse may open up the peripheral bed into the tissues rather than shunt easily by the tissues. This now has become the subject of a further study.

Following this series of experiments we feel that this method may provide a simple and safe way of handling surgical procedures in small children and infants in which an open right ventricle or atrium is desired.

SUMMARY

1. Ten dogs have undergone right ventriculotomy using an extracorporeal circulation from a reservoir of oxygenated blood in a system free of mechanical pumps and oxygenators.

2. Nine of these dogs survived the procedure, one died 4 weeks after operation from peritonitis with perforation of the small bowel.

3. Slow oxygenation of the returning caval blood permitted extension of the period of cardiac occlusion beyond that allowed for in the oxygenated reservoir of blood.

4. There has been no evidence of postoperative bleeding problems or other evidence of blood trauma.

5. Clinical application is proposed on the basis of simplicity and safety of the method for small children and infants where intracardiac procedures are desired.

REFERENCES

1. Wiggers, C. J.: Physiology of Shock. New York: Oxford Univ. Press, 1950.
2. Callaghan, J. C., and Delarue, N. C.: Sixty-seven minutes of ventricular fibrillation with survival. Unpublished data.
3. Brock, Sir Russell: Resuscitation and survival from two hours of cardiac arrest. Personal communication.
4. Stephenson, H. E., Corsan Reid, L., and Hinton, W.: Some common denominators in 1200 cases of cardiac arrest. Ann. Surg. *137*: 731-742, 1953.
5. Andreason, A. T., and Watson, F.: Experimental cardiac surgery. Brit. J. Surg. *39*: 548-551, 1951.
6. Warden, H. E., Cohen, M., Read, R., and Lillehei, C. W.: Controlled cross circulation for open intracardiac surgery. J. Thorac. Surg. *28*: 331-343, 1954.
7. Warden, H. E., DeWall, R. A., Aust, J. B., Ziegler, N., Read, R. C., Varco, R. L., and Lillehei, C. W.: Controlled total body arterial perfusion for open intracardiac surgery. J. Thorac. Surg. In press.
8. Kay, E. C., Cross, F. S., and Zimmerman, H. A.: Evaluation of techniques for repair of auricular and ventricular septal defects. J. Thorac. Surg. (In press.)

THE EXPERIMENTAL PRODUCTION OF PULMONARY VASCULAR DISEASE*

J. Francis Dammann, Jr., Rodney L. Smith and William H. Muller, Jr.

There are 2 major hypotheses concerning the etiology of pulmonary arteriosclerosis. In the first hypothesis, thrombo-embolization is considered the exciting factor. Experimentally, Duguid[1], Harrison, and others[3] have produced significant vascular changes in the pulmonary arteries of animals subjected to repeated intravenous infusions of finely broken-up blood clots. By sacrificing animals at suitable periods of time, all stages of transition from typical thrombi to the end arteriosclerotic lesion can be found. Similar transition stages have been described in patients otherwise normal or suffering from congenital heart disease[4,5]. Recently, O'Neal and Thomas[6], after a careful review of autopsy material, suggested that the frequent association of thrombi and arteriosclerosis in all types of congenital heart disease indicates that thrombi may be the initiating factor.

Equally impressive evidence has been accumulated suggesting that hypertension is the exciting factor in the production of both systemic and pulmonary arteriosclerosis. The repeated rapid injection of saline into the carotid artery of the rat produced systemic changes typical of malignant hypertension[7]. Stenosing a renal artery prevented change in that kidney by reducing the pressure impact of the injected saline. Comparable changes were produced by Kawakami[8] in the pulmonary arteries of rabbits using repeated injections of adrenalin.

This study was undertaken to help resolve the question of the etiology of pulmonary vascular disease.

METHOD

This study was divided into three parts.

I. In mongrel dogs, as large an anastomosis as possible was made between the distal end of the left main pulmonary artery and the side of the aorta. The majority of dogs died within 24 hours due to cardiac failure. Those dogs which survived were re-operated upon periodically so that pulmonary artery and aortic pressures and biopsies from both lungs might be obtained.

II. Anastomoses were made between the distal end of the left upper lobe pulmonary artery and either the subclavian artery or the side of the aorta. Pressures were obtained both before and after the anastomoses. Serial biopsies were taken from the left upper lobe and the left lower lobe.

III. A procedure similar to group II was carried out, but in addition, stripping of the vagus nerve or a complete denervation of the lung was attempted. Serial pressures and biopsies from the left upper and left lower lobes were obtained over a 1 year period.

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This work was supported in part by United States Public Health Grant Number H2038.

RESULTS

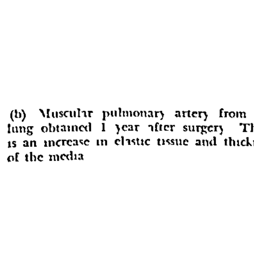
Group I. Only 5 of 21 dogs survived beyond 1 week because of irreversible cardiac failure. In general the severity of failure and also pulmonary vascular change was directly related to the size of the anastomosis. Vascular change was gradual and consisted of progressive hypertrophy of the small muscular arteries and the appearance after many months of intimal proliferation in small and large arteries.

Failure and pulmonary vascular change was most marked in the dog with the largest anastomosis. Pressures obtained at the time of surgery from the pulmonary artery were 120/75, whereas aortic pressure was 155/85. This animal was sacrificed 1 year after surgery. The anastomosis measured 8 mm. in diameter. The left ventricle and left auricle were greatly hypertrophied and dilated. A comparison of pre- and postoperative biopsies revealed a striking decrease in lumen diameter/wall thickness ratio. The small muscular arteries of the left lung were narrowed due to medial hypertrophy and occasional areas of intimal proliferation. No emboli or



(a) Muscular pulmonary artery from left *lung obtained at the time of completion of* an anastomosis between the aorta and left main pulmonary artery.

Fig. 1.



(b) Muscular pulmonary artery from left lung obtained 1 year after surgery. There is an increase in elastic tissue and thickness of the media.



thrombi were noted There was thickening of the walls of veins and capillaries In contrast there was less arterial change in the right lung but a comparable amount of venous change

Although absolute measurements of pulmonary blood flow were not made the appearance of severe irreversible left heart failure indicates a very large blood flow through the anastomosis into the left lung The

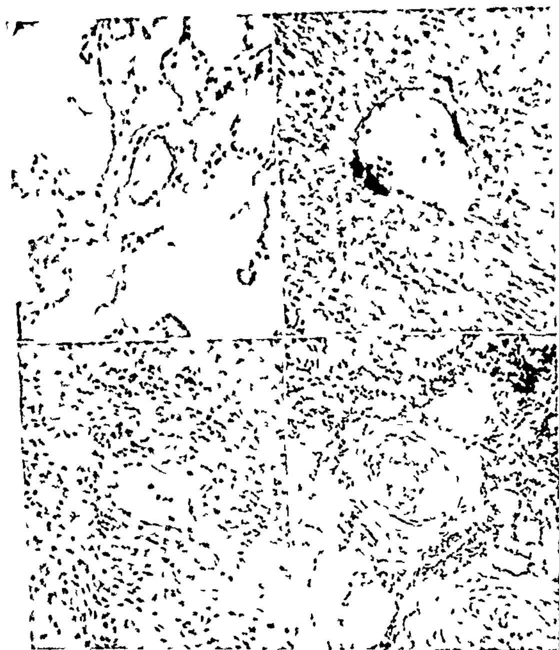


Fig 2 (a) *Upper Left* Small pulmonary artery from left lower lobe obtained after completion of an aortic left upper lobe pulmonary artery anastomosis

(b) *Upper Right* Small pulmonary artery from left upper lobe obtained at the time of operation There is gross perivascular hemorrhage

(c) *Lower Left* Small pulmonary artery from left upper lobe taken 6 months postoperatively There is a marked perivascular inflammatory response

(d) *Lower Right* Small pulmonary artery from left upper lobe obtained 17 months postoperatively The lumen has been occluded and the wall partially replaced by fibrous tissue

difference noted between the right and left lungs suggests that changes in the right lung were due to increased venous pressure resulting from chronic left heart failure, whereas changes in the left lung were due to chronic left heart failure plus increased pulmonary blood flow and increased pulmonary pressure.

Groups II and III. The second and third groups will be discussed together. There was no qualitative or quantitative difference in the response of the pulmonary vascular bed to high pressure between the group of systemic to left upper lobe pulmonary artery anastomoses and the group in which vagotomy and sympathectomy were also carried out. In no instance was there evidence of cardiac decompensation or dilatation and hypertrophy of the left side of the heart. Pressure studies revealed a uniform elevation of pulmonary artery pressure to a systolic level that was within 15 mm of mercury of the aortic systolic pressure.

Examination of the biopsies revealed an interesting evolution of changes. A typical sequence is shown in Fig. 2. Fig. 2a is a control photomicrograph of a small pulmonary artery taken from the left lower lobe at the time of surgery. The lumen is wide, media relatively thin and adventitia sparse. In this section the average lumen/wall ratio was 1:3. Fig. 2b is a photomicrograph of a vessel from the left upper lobe shortly after the anastomosis was opened. There is gross hemorrhage and the adventitia has been separated from the media by red blood cells. In some areas red cells were present in the media and intima. There were no clots. The lumen/wall ratio was 1:0. Fig. 2c represents a left upper lobe artery taken 6 months after the original operation. The lumen/wall ratio at this time was 3:1. No thrombi were seen. Around and in the wall of the vessel the red blood cells have been replaced by white cells and spindle fibroblasts. Fig. 2d represents a small muscular left upper lobe pulmonary artery obtained 17 months after the original operation. The lumen/wall ratio was 1:1. There has been a complete replacement of the cellular perivascular exudate and of the lumen with fibrous and hyaline material.



Fig. 3. Small and medium sized pulmonary artery from left upper lobe of dog 8 months after an aortic left upper lobe pulmonary artery anastomosis. The small artery is a branch of the larger vessel.



Fig 4 Granulomatous appearing lesion of proliferative endothelium involving a small pulmonary artery from the left upper lobe of a dog 1 year after an aortic left upper lobe pulmonary artery anastomosis

A study of serial sections taken from the same segment of lung revealed 2 additional significant changes Following along the course of a medium sized pulmonary artery the most marked change was usually found where the vessel bifurcated Where the lumen was almost obliterated the vessels arising distal to this obstruction appeared essentially normal The vessel lying to the left in Fig 3 is a branch of the larger artery to the right The vessel on the right is almost completely obliterated whereas the artery on the left has a large lumen and relatively thin media This suggests that obstruction of a vessel at one point may protect the vessel at a more distal point

In some areas granulomatous appearing lesions were found (Fig 4) These appeared as masses of moderately large cells with many spaces lined by endothelial cells and containing red blood cells The original architecture of the artery was hard to distinguish These lesions were arterial and appeared to be located immediately distal to areas of acute arteritis They resemble in structure the lesions found in patients with a reverse patent ductus arteriosus[9] and noted in a few instances of primary pulmonary hypertension [10]

DISCUSSION

Serial biopsies of the affected lung of animals subjected to a sudden increase in pressure in the left upper lobe demonstrate a definite progressive pattern of pulmonary vascular change This developmental sequence lends strong support to the simple mechanical explanation of pulmonary vascular disease The sudden increase in pressure brought about by anastomosing a systemic vessel to a small portion of the pulmonary arterial bed leads to overstretching of the arterial walls The greatest distention occurs at the point of bifurcation of a medium sized pulmonary artery into a small muscular artery Overstretched muscle undergoes necrosis Plasma and blood seep into this weakened area and an inflammatory response results The inflammatory response and the healing process vary depending upon the rapidity of destruction of the vessel wall Most frequently there is a

complete replacement of the vessel wall by a poorly organized condensation of fibrous tissue elements Muscle and elastic fibers are absent or fragmented The lumen is replaced by a connective tissue mass interwoven with a few small capillary channels This response is the most common However in several instances the principal reaction appeared to be proliferative As suggested by Edwards[11] the hyaline and fibrinoid material *in the vessel wall was irritative to the surrounding endothelium and caused* a proliferation of endothelium of a villous character Clumps of endothelial cells were separated by blood spaces filled with red blood cells These lesions have been termed arteriovenous fistulae[1] congenital defects and the end result of a recanalized thrombus[9] Serial sections through such areas demonstrate that they are entirely arterial, do not connect with a vein and appear most frequently near the origin of the small muscular pulmonary arteries

The contrast in the response of the pulmonary vascular bed when the anastomosis was to the total left lung and when it was to a small portion of the left lung suggests that the end result varied directly with the degree of stress In group I pulmonary pressure was elevated but did not approach systemic Vascular changes consisted of hypertrophy of the media followed by gradual and spotty intimal proliferation In groups II and III pulmonary artery pressure was sharply elevated to systemic levels The response was an acute arteritis followed by gradual healing with replacement of individual arteries by recanalized masses of connective tissue or a granulomatous type of endothelial lesion The parallel to essential and malignant systemic hypertension is inescapable Systemic arteries and arterioles are narrowed by medial hypertrophy and intimal proliferation as a result of or a cause of chronically elevated systemic pressure Necrotizing arteriolitis results from or causes malignant hypertension The evidence from this experimental work suggests that pulmonary vascular change is a result and not a cause of hypertension

CONCLUSIONS

1 Anastomosis of the left pulmonary artery to the aorta produces a rise in pressure and blood flow in the left lung There is a gradual hypertrophy of the media of the muscular pulmonary arteries followed by spotty intimal proliferation

2 Anastomosis of a systemic vessel to the distal left upper lobe pulmonary artery causes a much greater response An acute arteritis involving the small muscular pulmonary artery results Healing occurs gradually either by replacement of the affected arteries with fibrous mass embracing a few recanalized areas or by a proliferative group of endothelial cells and blood spaces

3 *In this experiment there was no evidence of thrombi except in the* late healing stage

4 Small arteries distal to an area of focal arteritis may remain normal since the stress of a high pressure is not placed on them

5 Vagotomy and sympathectomy do not appear to offer any protection to the lung subjected to a sudden high pressure

6 There appears to be a marked similarity between the changes observed in this series and those noted in essential and malignant hypertension

REFERENCES

1 Duguid J B The arterial lining Lancet Lond 2 207 1952
2 Harrison C V Experimental pulmonary arteriosclerosis J Path Bact Lond 60 2 1948
3 Muirhead E E and Montgomery P O B Thrombo embolic pulmonary arteritis and vascular sclerosis Arch Path Chic 52 505 1951
4 Heard Brian E An experimental study of thickening of the pulmonary arteries of rabbits produced by the organization of fibrin J Path Bact Lond 64 13 1952
5 Rich A R A hitherto unrecognized tendency to the development of widespread pulmonary vascular obstruction in patients with congenital pulmonary stenosis (Tetralogy of Fallot) Bull Johns Hopkins Hosp 82 389 1948
6 O Neal R M and Thomas W A The role of pulmonary hypertension and thromboembolism in the production of pulmonary arteriosclerosis Circulation N Y 12 370 1955
7 Byrom F B and Dodson L F The causation of acute arterial necrosis in hypertensive disease J Path Bact Lond 60 357 1948
8 Kawakami I Experimental arteriosclerosis in the lung especially on research by reconstructing model Part I Adrenalin arteriosclerosis Nippon J Cl Angiocardiology 13 269 1950
9 Damman J F Jr Berthrong M and Bing R J Reverse ductus A presentation of the syndrome of patency of the ductus arteriosus with pulmonary hypertension and a shunting of blood flow from pulmonary artery to aorta Bull Johns Hopkins Hosp 92 128 1953
10 Symmers W St C Necrotizing pulmonary arteriopathy associated with pulmonary hypertension J Clin Path, Lond 5 36 1952
11 Edwards J E Pathologic considerations in adjustments between the systemic and pulmonary circulations Cardiovascular Surgery Henry Ford Hosp International Symposium Philadelphia W B Saunders Co 1955
12 Taussig H B Congenital malformations of the heart New York The Commonwealth Fund 1947

EXPERIMENTALLY PRODUCED PULMONARY VALVULAR STENOSIS*

ANGELO RIBERI PAUL F GRICE AND WARREN E COGGESHALL

A number of authors have attempted to study the problem of pulmonary stenosis experimentally Loose ligatures have been placed around the pulmonary arteries of puppies with resultant stenosis as full growth is attained[1] and the same result has been achieved by resecting a portion of the wall of the artery[2] In a similar manner infundibular stenosis has been created[3,4] The only attempt to produce pulmonary valvular stenosis has been by cauterization of the valve cusps and the commissures with fuming nitric acid These methods do not reduplicate the condition as seen in patients with congenital fusion of the cusps

It was the purpose of this study to bring about pulmonary valvular stenosis by direct suture of the valve leaflets and to study the resultant hemodynamic changes in animals with such lesions and in others in which an atrial septal defect was created in addition

*From the Indiana University School of Medicine Indianapolis Indiana Aided by Grants from the Indiana Heart Foundation the United States Public Health Service the James Whitcomb Riley Memorial Association and by a contract between the Office of Naval Research the United States Navy and Indiana University

MATERIAL AND METHOD

Healthy mongrel dogs were anesthetized by the intravenous administration of thiopenthal sodium, intubated and hyperventilated with oxygen by an intermittent insufflation apparatus. They were cooled in a bath of water and crushed ice to a rectal temperature of 30° C. Exposure was obtained through a complete sternal splitting incision. The pericardium was opened and the right atrial superior vena caval area was infiltrated with procaine until the characteristic slowing of the heart rate and alteration of electrocardiographic P waves were noted.[5] By this time the rectal temperature had fallen to from 26.5° to 28° C. During complete venous inflow occlusion the pulmonary artery was incised transversely 2 cm. above the valve ring. The 3 commissures were rapidly closed partially by one or two 4-0 arterial sutures passed through the valve leaflets and tied (Fig. 1). The incision in the pulmonary artery was repaired with an everting mattress suture of 5-0 silk. The superior vena caval occlusion was released 2 or 3 minutes before the inferior vena caval clamp. Good cardiac contractions followed a brief period of massage and the injection into the right ventricle of 1 or 2 cc. of 1 to 20,000 epinephrine.

A systolic pulmonary thrill was palpable in each animal. Most of the animals were subjected to cardiac catheterization. In some animals an atrial septal defect was created either before or after production of valvular stenosis by open cardiotomy without hypothermia.[6] These animals were subsequently studied by cardiac catheterization.

RESULTS

All animals had a pulmonary systolic murmur judged to be grade II or III. Seven of the 19 animals in which valvular stenosis was created died from right heart failure in from 2 to 62 days and showed at postmortem examination ascites, pleural effusion and passive congestion of the liver.

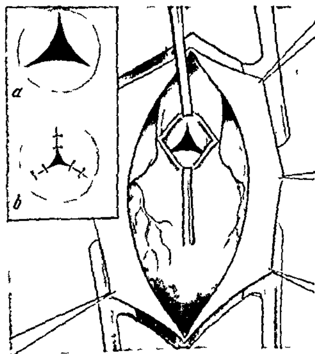


Fig. 1. Sketch of the operative procedure showing in insert (a) the appearance of the valve before and in (b) after suture approximation of the commissures.

Table 1 Data Concerning Dogs with Experimentally Produced Pulmonic Valvular Stenosis

NO OF DOG	TEMP IN DEGREES C	TIME OF CAVAL OCCLUSION IN MIN	STITCHES IN EACH COMMISSURE	ATRIAL SEPTAL DEFECT	GRADE OF SYSTOLIC MURMUR	FINAL RESULT
570	27 5	11	1	none	[illegible]	Sacrificed after [illegible] months
571	27 5	8	1	none	3	Dead 11 days heart failure ascites pleural effusion
574	26 8	9	1	none	3	Dead [illegible]0 days Cause unknown
584	28	10	1	1 month prior to the stenosis	3	Dead 1 week right heart failure ascites pleural effusion
592	26 5	10	1	none	3	Sacrificed after [illegible] months
596	26.5	8	1	none	3	Sacrificed after 6 months
21	27 5	8.5	1	2 months after stenosis	3	Sacrificed after 3 months
22	26 5	8	1	none	3	Dead 62 days right heart failure ascites liver congestion edema
35	27	6.5	1	none	3	Dead 60 days right heart failure ascites pleural effusion
43	27 6	7	1	none	3	Dead 30 days right heart failure ascites pleural effusion
58	27	6.5	1	1 month prior to the stenosis	3	Sacrificed after 1 month insufficiency
90	27.5	8	2	2 months after stenosis	2	Sacrificed after 3 months.
45	26	6.5	2	none	2	Dead [illegible] days right heart failure ascites pleural effusion
92	26.5	7	2	1 month after stenosis	2	Sacrificed after 3 [illegible] days
99	27	6.5	2	none	2	Sacrificed after 3 months.
100	27	8	2	none	2	Dead 4 days, right heart failure ascites pleural effusion
100ND	26 8	7	2	15 days after stenosis	2	Sacrificed after 3 months
121	27.5	6.5	2	2 months after stenosis	3	Sacrificed after 6 months.
635	28	8	1	1	3	Sacrificed after 3 months

Table 2 Catheterization Data

NO OF DOG	PRESSURE IN MM HG				VOL PER CENT O_2							PER CENT SATURATION ARTERIAL BLOOD	SHUNT	
	WEDGE	MPA	RV	RA	HC	SVC	RA	RV	MPA	FEM sO_2	ART cO_2		R→L	L→R
ANIMALS WITH PULMONIC VALVULAR STENOSIS														
574		48/20	86/9											
596		32/14	56/3											
635		18/11	39/6											
21		16/9	44/5	15										
21 repeat		18/8	49/7	0										
22		23/15	33/7 5	1 1										
3[illegible]		17/13	38/7	1										
43		17/10	35/5	1										
90	2	21/11	48/7	1										
92	10	31/17	81/10	7 1										
99		21/10	51/16	4.5										
101	9	28/14	10[illegible]/17	8										
121	5	23/6	132/8	4										
WITH ATRIAL SEPTAL DEFECT														
58		31/8	31/6	4	11 4	11 1	10.2		9 6	14 9	19 9	97%	O	O
90		30/9	68/5	6	9	9 5	9		9 5	10 6	13 7	96%	O	O
92		30/12	59/1	4	9 5	9 5	9 3		9 1		15 8	91%	✓	O
100		35/20	130/21	20	3 8	5 7	4		5	7 7	10.5	104%	O	O
121		23/5	105/3		5	5 8	5		5	9 6	11 4	81%	✓	O



Fig 2 Photograph showing the stenotic valve in dog Number 121 six months after operation and for comparison a normal pulmonary valve

(Table 1) One died of unknown cause The remainder were sacrificed in from 1 to 6 months The valve area was judged to have been reduced by approximately one third in those animals in which 1 suture had been placed and by two thirds in those with 2 sutures in each commissure

Catheterization data are recorded in Table 2 In all animals with pulmonic stenosis alone a significant increase in right ventricular pressure was noted as compared with pulmonary artery pressure and this was true in all but one in which an atrial septal defect was also present This exceptional animal was the only one in which a stitch in 1 commissure had pulled out with production of insufficiency The maximum pressure difference noted was in dog Number 121 (Fig 2) with a pulmonary artery pressure of 23/6 and a right ventricular pressure of 132/8 In those dogs with a single suture in each commissure the pulmonary artery pressure averaged 23 7/12 3 and the ventricular pressure 17 1/6 1 These average values in animals with 2 sutures in each commissure were 25 1/11 6 and 103 1/11 6 respectively A right to left shunt was apparent at rest in 2 of the 5 dogs studied with combined defects

Four animals had measurements of peripheral venous pressures before and after running for 10 minutes at 3 m p h on a treadmill inclined 15° The pressure increased from 7 to 25 cm of water in the first from 8 to 25 in the second from 22 to 16 in the third and from 25 to 50 in the fourth (Dogs 21 90 100 and 121) In 2 dogs (Dogs 100 and 121) in which the resting arterial oxygen saturation was 101 and 81 per cent the values after exercise dropped to 72 and 60 per cent respectively

SUMMARY

By suturing the pulmonary valve cusps together at the commissures pulmonary valvular stenosis remarkably like that seen in patients was created in dogs In some an atrial septal defect was also produced All but one of the animals which died showed evidences of severe right heart failure Cardiac catheterization revealed significant elevation of right ventricular pressure over that in the pulmonary artery A right to left shunt was

demonstrable in 2 of the 5 animals with combined lesions studied by catheterization Even when a shunt was not noticeable at rest it was evident with exercise Peripheral venous pressures in both groups of dogs rose with exercise

REFERENCES

1 Holman E Hemicardiac hypertrophy due to increased peripheral resistance A study of pulmonic and aortic stenosis experimentally produced J Thorac Surg 9 262 1940
2 Hufnagel C A Roe B B and Barger A C A technique for producing pulmonary artery stenosis Surgery 29 77 1951
3 Alden J F Haddy F J Adams W L and Baronofsky I D Cardiodynamics of experimental infundibular stenosis in Surgical Forum 1952 Philadelphia W B Saunders Co 1953 pp 299 304
4 Sabiston D C and Williams C R The experimental production of infundibular pulmonic stenosis Ann Surg 139 325 1954
5 Riberi A Siderys H and Shumacker H B Jr Ventricular fibrillation in the hypothermic state I Prevention by sino auricular node blockade Ann Surg (in press)
6 Moore T C and Shumacker H B Jr Experimental Creation of Atrial Septal Defects with some notes on the production of a right to left atrial shunt Angiology 4 244 1953

EVALUATION OF A MECHANICAL SHUNT TO BYPASS SEGMENTS OF THE THORACIC AORTA INCLUDING THE ARCH*

FREDERICK S CROSS YOICHI HIROSE RICHARD D JONES
STEPHEN S HUDACK AND EARLE B KAY

Techniques have been described previously to assure adequate blood to or protection of areas distal to the site of total occlusion of the thoracic aorta Johnson and Kirby have used large caliber polyethylene shunts Stranahan and associates[3] heterologous aortic grafts while Cooley and DeBakey[1] rely on the protective effects of hypothermia Such methods have been criticized however because of the bulkiness and difficulty of insertion of large plastic shunts the necessity of at least two extra anastomoses and subsequent repair of vessels when heterologous or homologous grafts are used and the risks inherent in the use of hypothermia especially in older people as well as the danger of aortic arch hypertension present when shunts are not used

It is the purpose of the present study to eliminate such disadvantages through the use of small caliber flexible tubing in association with a pump as a bypass system the small catheters to assure ease of insertion and removal the pump to maintain adequate blood rates through the small tubing

METHOD

Three series of experiments with 7 dogs in each series were carried out each utilizing slightly different bypass routes (Fig 1)

*From the Surgical Research Laboratories of St Lukes Hospital Cleveland Ohio Supported in part by the Prentiss Foundation and by Research Grant from the Cleveland Area Heart Society

I Left subclavian artery to left femoral artery for bypass of the descending thoracic aorta

II Left ventricle to left femoral artery for bypass of the ascending aorta

III Left ventricle to left femoral artery and right innominate artery for complete aortic arch isolation

In healthy mongrel dogs anesthetized with sodium pentobarbital 25 mg per kg and heparinized with 3/4 mg per pound a thoracotomy incision is made through the left fourth or fifth interspace after previously isolating both femoral arteries through small groin incisions The entire aortic arch from the base of the heart to the first intercostal artery is dissected free including the innominate and left subclavian branches The subclavian and femoral arteries are cannulated with Number 12 or Number 14 French expandable nasal oxygen catheters the subclavian catheter being equipped with an accessory tube to be attached to a mercury manometer for blood pressure recordings in the aortic arch In series II and III the dog's left ventricle is cannulated by way of the left atrial appendage with a specially constructed J shaped tygon tube (1/16 inch I D) with multiple openings at its intake end and with a smaller tube incorporated for continuous recording of ventricular pressures The ventricular catheter is J shaped so that when inserted the long arm of the J lying outside the heart arches toward the cardiac apex and is completely out of the operative field

The afferent limb of the shunt (tygon tubing 7/16 inch I D) carries blood to a graduate cylinder reservoir previously primed with 500 cc of heparinized whole blood (25 mg per 500 cc) From the reservoir blood is pumped through the efferent limb of the shunt to the dog's femoral artery with a model T 56 Sigmamotor pump A negative pressure of 50 mm of Hg is maintained in the reservoir from a wall vacuum source Blood flow to and from the reservoir can be controlled independently the inflow by a screw clamp on the afferent limb of the shunt and the outflow by changes in pump speed Blood transfusions to the dog for measured blood loss replace

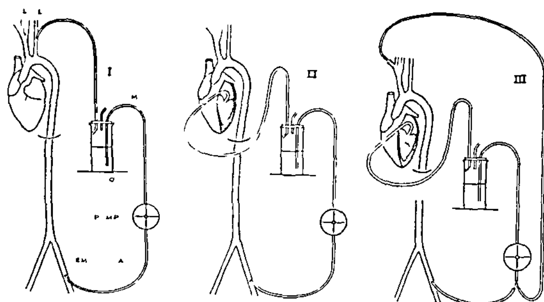


Fig 1 Diagrammatic sketch of shunt mechanism with the different shunt routes used in the three series of experiments shown

ment are pumped from the reservoir Blood pressures above and below the aortic occlusion site are obtained continuously with mercury manometers attached to a small catheter in one femoral artery and to the small tube incorporated in the inflow catheters as described previously

The pump is started prior to cross clamping the aorta at a rate that is estimated to be adequate on the basis of the dog s weight since a rough correlation was found between the two For example dogs weighing 30 pounds required a pump output of 500 to 700 cc per minute In series III in which the cerebral and peripheral circulation is delivered through separate catheters 15 to 20 per cent of the pump output goes to the catheter in the innominate artery to maintain cerebral circulation and the remainder goes to the periphery by way of the femoral artery catheter

The aorta is then cross clamped at the desired level and inflow outflow balance in the reservoir obtained at a pump speed sufficient to prevent hypertension proximal to the clamp i e in the aortic arch or left ventricle and to maintain an adequate femoral artery pressure distal to the clamp Obviously pump speeds are more critical when shunting directly from the left ventricle than from the left subclavian artery When the circulation through the shunt is correctly balanced it can be continued for long periods of time with only minor adjustments in the pump flow rates Any blood loss or peripheral vaso dilatation leading to a blood pressure drop is automatically compensated for from blood in the reservoir

Continuous aortic occlusion times ranged from 15 to 60 minutes At the conclusion of the bypass procedure the clamps are taken off the pump stopped and the catheters removed Protamine sulphate in amounts sufficient to lower the clotting time to from 5 to 10 minutes is given The chest is closed with interrupted silk technique and then aspirated until free of residual air and blood No special postoperative treatment other than this has been necessary

RESULTS

Early experiments were directed at perfecting the mechanical aspects of the problem and mortality was high Following this it was possible to have 16 consecutive dogs survive the shunting procedure without evidence of *neurological sequelae*

Femoral artery pressures distal to the aortic occlusion site were maintained above 80 mm of Hg and occasionally were as high as 110 mm of Hg Arch and ventricular pressures proximal to the site of aortic occlusion were usually held slightly above or slightly below pre aortic occlusion levels In series I it was possible to vary the blood pressure above and below the site of occlusion simply by varying the pump speeds With increased speeds arch pressure dropped and peripheral pressure increased while with decreased speeds the opposite was obtained (Fig 2) In series II and III whereas this was also possible we were more interested in maintaining a steady ventricular pressure at pre aortic occlusion values since large changes in pressure above and below this control level led to cardiac irregularities and occasionally to cardiac arrest

Fourteen dogs in series I and II survived the shunting procedure without neurological sequelae In series III 3 of 7 dogs (13 per cent) died with evidence of cerebral damage within 8 hours of the conclusion of the experiment 1 with proven cerebral air emboli, the others presumably from air or

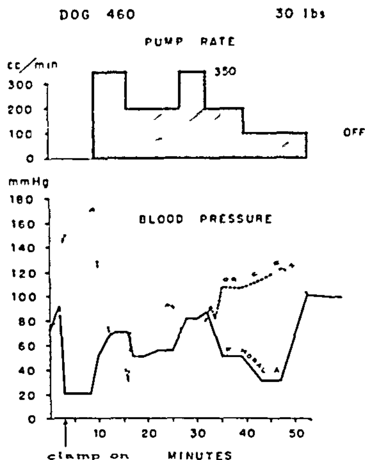


Fig 2 Graph illustrating the changes in arch and femoral artery pressures associated with changes in pump speeds in series I dogs Note the marked hypertension in the arch from cross clamping the descending aorta without the shunt functioning



Fig 3 The excellent exposure of aortic arch area with a series III shunt in place is shown The intake catheter in the left ventricle is completely out of the operative field

A Ventricular shunt
B Aortic Arch

fibrin emboli in the cerebral circulation It was felt however that with additional experience in a larger series this mortality could be reduced significantly

At the conclusion of the experiments systemic blood pressures were close to the pre experiment level In other words peripheral vascular tone was maintained through the long period of bypass circulation with the pump Occasionally early in the shunt procedure there was evidence of a temporary

vasodilatation which necessitated increased pump speeds. Later as vascular tone returned, blood pressure rose and the pump could be slowed.

Exposure of the aortic arch was unobstructed with the shunts in place as shown in Fig. 3.

DISCUSSION

The shunt in series I was utilized in resections of the descending aorta; that in series II afforded a means for inserting an arch graft in a retrograde manner, first anastomosing a distal end of the graft to the side of the descending aorta and concluding with an end-to-end anastomosis between the proximal graft and ascending aorta. The shunt was necessary only during the last anastomosis. The series III type shunt allowed complete occlusion and resection of the aortic arch while peripheral and cerebral circulation were being maintained.

The shunting procedures were simple and mechanically satisfactory. It was possible to complete the insertion of the intake and outflow catheters in about 10 to 15 minutes longer than it took to do the basic dissection of the aortic arch area. Likewise, the removal of the catheters was simple. A possible criticism of the technique is the necessity for the use of heparin but this has not been found to be a serious drawback to date. It has been possible utilizing series II type shunts to carry out entire aortic arch excisions with replacement of homologous grafts.

SUMMARY

1. Mechanical shunts of small caliber tubing for ease of insertion and removal in conjunction with a Sigmamotor pump to obtain adequate blood flow rates have been described to bypass segments of the thoracic aorta including the arch.

2. By utilizing such shunts occlusion of the descending and ascending thoracic aorta has been tolerated for 15 to 60 minutes without mortality or neurological sequelae.

3. Complete aortic arch occlusion as in series III dogs was less well tolerated, 3 of 7 dying of cerebral damage.

4. It has been possible to replace segments of thoracic aorta including the entire arch with the aid of these shunting mechanisms.

REFERENCES

1. Cooley, D. A. and DeBakey, M. E.: Resection of the thoracic aorta with replacement by homograft for aneurysms and constrictive lesions. J. Thorac. Surg. 29: 66-104, 1955.
2. Johnson, J., Kirby, C. K., Lehr, H. B.: A method of maintaining adequate blood flow through the thoracic aorta while inserting an aorta graft to replace an aortic aneurysm. Surgery 37: 54-57, 1955.
3. Stranahan, A., Alley, R. D., Sewell, W. H. and Kausel, H. W.: Aortic arch resection and grafting for aneurysm employing an external shunt. J. Thorac. Surg. 29: 54, 1955.

A VASCULAR ANASTOMOSIS CLAMP ALLOWING RAPID RECONSTITUTION OF BLOOD FLOW*

WILLIAM D KELLY AND JOHN J ALDEN

Vascular surgery is still in need of a technique which will allow the rapid coupling of blood vessels while permitting the use of the standard suture techniques which have proved highly successful The resection and grafting of lesions in the thoracic aorta and carotid vessels are areas in which the need is most critical Experimental work dealing with homografting would be also facilitated by such a method Attempts have been made to handle this problem by the use of intraluminal and extraluminal shunts and hypothermia[1, 2, 3, 4, 5] These procedures have been only partially successful and frequently add considerably to the difficulty and hazard of the operation The present report deals with the use of a new clamp which allows the rapid coupling of blood vessels with early reconstitution of blood flow at the same time allowing the use of conventional suture technique

METHOD

The vascular anastomosis clamp (Fig 1a) is made entirely of stainless steel† Jaws in the form of a split ring with a projecting arm fit into the slotted end of handles which can be rigidly interlocked The jaws are hinged at the end opposite the projecting arms and have fine teeth similar to those used in the Potts clamp Short segments of metal tubing soldered on the side receive a fine pin equivalent in diameter to a 21 gauge needle Various size jaws are available Only 2 sizes 7 mm and 9 mm in diameter were used in this study Figure 1b c d e f illustrates the fundamental technique Air is washed from the vessel by releasing the peripheral occluding forceps just as the everted vessels are coupled by interlocking the handles The handles are approximated using the index finger and thumb to estimate the amount of pressure being applied on the everted ends The turnbuckle is then tightened just to this point Blood flow is then reconstituted by releasing the proximal occluding clamp

Grafts are easily inserted by the use of 2 clamps the graft being mounted on one half of each set The other halves are applied to the severed ends of the recipient vessel and the graft then simply inserted and coupled to the appropriate handles (Fig 1f)

PROCEDURE

Adult mongrel dogs were operated upon under sodium pentobarbital anesthesia using standard sterile technique and positive pressure mechanical respiration Through the bed of the left fifth rib the first portion of the descending thoracic aorta was mobilized for a distance of 6 to 8 cm dividing 3 to 4 pairs of intercostal arteries Either division and anastomosis or resection and insertion of a homograft were then carried out excising 2 to 4 cm of aorta in the latter case The grafts averaged 4 5 cm in length Anastomoses were performed with 5 0 arterial silk using continuous whipping or

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†Machine work by Oliver Moe 1626 Hartford Avenue St Paul Minnesota

everting stitches Penicillin 300 000 u was given daily for 5 days after operation

Homografts were obtained from recently dead animals Initially no attempt was made to process the grafts properly most of them simply being placed in a deep freeze until used at which time they were thawed out with warm water and placed in zephiran for 15 to 20 minutes Several non sterilized grafts kept in Ringer s solution in the ice box for a number of days are also included in this group These grafts were used in the initial experiments designated series A simply to test the anastomotic clamp prior

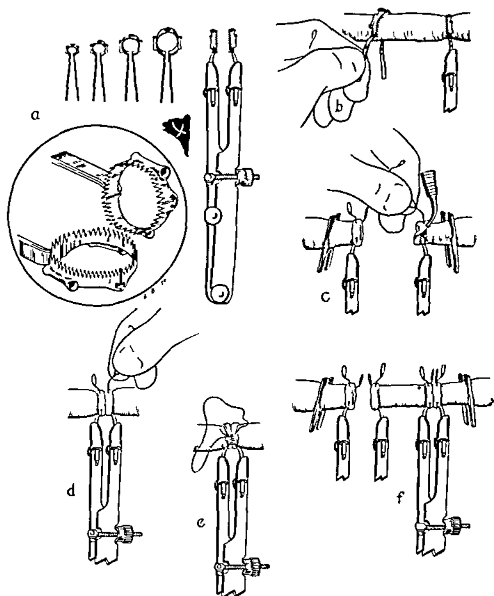


Fig 1 (a) A vascular anastomosis clamp allowing rapid reconstitution of blood flow (b) Jaws being placed around intact vessel (c) Blood flow occluded vessel divided ends being everted and fixed in this position with fine pins (d) The everted ends are coapted the handles have been interlocked the occluding clamps have been removed allowing blood flow to resume The pins are being removed (e) The everted edges are sutured using standard techniques Following complete circumferential anastomosis the handles are unlocked and the jaws removed from around the vessel (f) The use of 2 sets of clamps allows the insertion of homografts by the same technique

to setting up facilities for processing and storing grafts according to acceptable standards.

Series B designates those experiments also performed consecutively in which homografts were processed by sterilization with beta propiolactone and stored at 4 C in buffered polyionic plasma solution as described by Cross *et al*.*

All animals were carefully observed after operation for hindquarter paralysis or weakness. After survival for several months a number of the animals were used in other nonrelated experiments. Some animals were sacrificed in the early postoperative period to observe healing. Specimens removed at autopsy were photographed and sections taken for histologic examination using both the hematoxylin and eosin stain and the Verhoeff elastic fiber stain routinely. Animals alive as of this writing have been evaluated by palpation of the femoral pulse and by aortography.

RESULTS

The immediate results are presented in Table I. Eversion and pinning of the blood vessels on the jaws of the clamp is no problem if the diameter of the jaw is roughly equal to the size of the collapsed vessel. Considerable leeway was found to exist, however. The couplings are water tight. In no instance did a vessel slip out of the jaws during anastomosis. Suturing is easily performed. Little or no anastomotic line bleeding occurs after removal of the jaws. No gross damage is apparent on the vessel wall where

Table I. Early Results Following Use of a Vascular Anastomosis Clamp

GROUP	NO.	DURATION OF TOTAL BLOOD FLOW OCCLUSION	DEATHS AT OPERATION	POSTOPERATIVE PARALYSIS OR WEAKNESS	DEATHS IN POSTOP. PERIOD
I Division and Anastomosis of descending thoracic aorta	22	Average = 2 45 (Range 1 45 — 3 30")	0	0	4* { 3 hemorrhage from anastomosis; 1 Empyema
II Homograft into thoracic aorta					
Series A (Inadequately processed grafts)	12	Average = 2 58 (Range 2 10 — 3 30")	0	0	5* { 1 hemorrhage from anastomosis; 1 hemorrhagic enteritis
Series B (Grafts Sterilized B Propiolactone stored in balanced polyionic solution)	17	Average = 3 12 (Range 2 00 — 5 00)	0	0	0

Discussed in text

Table 2 Late Results Following Use of a Vascular Anastomosis Clamp in the Thoracic Aorta

GROUP	NO SURVIVING POSTOP PERIOD	SACRIFICED	DIED AFTER USE IN OTHER EXPERIMENTS	ALIVE AND WELL
I Division and Anastomosis	18	3 (1 9 13 days p.o)	6 (89 218 days p o)	7 (195 285 days p.o)
II Insertion of Homograft				
Series A (Inadequately processed grafts)	7	2 (9 13 days p.o)	4 (85 195 days p.o)	1 (215 days p o)
Series B (Standard processing)	17	2 (Both at 7 days p o)	6 (93 125 days p o)	9 (108 215 days p o)

grasped by the clamp save occasionally for faint linear markings such as are commonly seen after the application of Pott s clamps Four animals sacrificed immediately after use of the anastomosis clamps showed no significant gross evidence of damage to the aorta

Of the 7 specimens removed from animals sacrificed during the first 2 weeks after operation none showed major damage either grossly or on microscopic examination Minimal intimal roughening was noted in 1 of these specimens One of these showed cleavage of the inner one third of the elastic fibers at one point adjacent to an anastomosis on microscopic examination

Two of the 8 specimens obtained from animals dying late after division and anastomosis of the thoracic aorta showed minimal defects One possessed a thrombus 1 0 mm in diameter in the suture line The other showed mild dilatation of the vessel at the anastomotic site with a loop of silk projecting into the lumen The 7 surviving animals all possess normal pulses and showed no significant abnormality on aortography

The 1 specimens removed from series A animals dying late all showed good healing of the anastomoses with no remarkable abnormalities

The 6 specimens obtained from series B animals dying late showed well healed smooth anastomoses in all

All of the specimens of homografts obtained from animals dying late showed some degenerative change with either calcium plaques thrombi or both being present However all except one of these lesions were located in the central portion of the graft whereas the areas adjoining the lines of anastomosis were free of notable defects

All 9 surviving animals in series B have normal femoral pulses Aortography in 8 of these dogs revealed only one significant abnormality consisting of an approximately 50 per cent narrowing of the lumen at the site of the distal anastomosis

COMMENT

The presence of a marked polymorphonuclear infiltrate through the wall of the vessels which disrupted following division and anastomosis suggests that infection may have played an etiological role. The frequent failure of inadequately processed homografts by disruption has been reported by others.* The safety of the clamp technique is best supported by the 17 consecutive operations without a death in which adequately processed homografts were used.

The uniform speed (average = 3 minutes) with which blood flow can be reconstituted falls within the limits during which total occlusion of the circulation can be tolerated even by the brain. At present experiments are under way to evaluate use of the clamp in replacement of the arch of the aorta in dogs. Apart from the necessity for speed in certain critical areas, it would seem theoretically more ideal to perform rapid coupling and restoration of blood flow in any area of the body as the vascular lumen is then not exposed to the air for prolonged periods, not to mention the possible secondary alterations in flow through the anastomosed vessel which may occur due to tissue edema as a consequence of anoxia.

One difficulty in the application of the clamp is the necessity of having an appreciable segment of host vessel to permit rapid eversion and fixing of the cut end on the jaws of the clamp. Recent improvements in the dimensions of the clamp jaws permit one to deal satisfactorily with a segment as short as 1.0 to 1.5 cm, depending on the diameter of the vessel. If one sacrifices a certain amount of the rapidity of application, it is possible to apply the clamp successfully on even shorter segments.

SUMMARY

A vascular anastomosis clamp is described which allows the temporary coupling of blood vessels and restoration of blood flow within an average time of 3 minutes. Conventional suture methods are then used to make the anastomosis. The method has been tested in 51 dogs and found to be simple, safe, productive of excellent anastomoses with little or no damage to the vascular wall.

REFERENCES

1. Carrel, Alexis: On the experimental surgery of the thoracic aorta and the heart. Ann. Surg. *52*: 83, 1910.
2. Hardin, C., Batchelder, T. L., and Schaefer, I. W.: The temporary use of polyethylene shunts in the resection and homologous graft replacement of the aortic arch in the dog. Surgery *32*: 219, 1952.
3. Johnson, J., Kirby, L. K., and Herndon, B. I.: A method of maintaining adequate blood flow through the thoracic aorta while inserting an aorta graft to replace an aortic aneurysm. Surgery *37*: 54, 1955.
4. Mahorner, H., and Spencer, R.: Shunt grafts. Ann. Surg. *139*: 439, 1954.
5. Izant, R. J., Hubay, C. A., and Holden, W. D.: A nonsuture aortic shunt—an experimental study. Surgery *33*: 233, 1953.
6. Pontius, R. G., Brockman, H. I., Hardy, E. G., Cooley, D. A., and DeBakey, M. E.: The use of hypothermia in the prevention of paraplegia following temporary aortic occlusion. Surgery *36*: 33, 1954.
7. Beattie, F. J., Adovasio, D., Keshishian, J. M., and Blades, B.: Refrigeration in experimental surgery of the aorta. Surg., Gyn., Obst. *96*: 711, 1953.
8. Gross, R. F., Bill, A. H., Jr., and Peirce, E. C.: Methods of preservation and transplantation of arterial grafts. Surg., Gyn., Obst. *88*: 689, 1949.
9. Gross, R. F., Hurwitt, E. S., Bill, A. H., and Peirce, F. C.: Preliminary observations on the use of human arterial grafts in the treatment of certain cardiovascular defects. N. England J. M. *239*: 578, 1948.

THE EXPERIMENTAL USE OF BRAIN PERFUSION IN OPEN CARDIAC SURGERY*

William H. Peniston and Victor Richards

A simple and practical method of total circulatory bypass to permit open intracardiac surgery is undoubtedly the goal of many ardent workers in the field of surgical research. Many notable contributions have been made and at present these may be classified in 3 broad categories: hypothermia, mechanical pump oxygenators and biological oxygenators. The concept of biological oxygenation has been attractive to the authors and accordingly a group of experiments were carried out to attempt the perfection of such a mechanism. Since total body cross circulation had been reported by others[1] and we felt entailed certain disadvantages, it was decided to attempt a regional type of cross circulation experiment. The most critical region of the circulation is the brain. We hoped therefore that by the simple expedient of brain perfusion with a donor circulation open cardiac surgery might be tolerated.

HISTORY

The concept of brain perfusion is not a new one. In 1920 Tuffier[2] suggested the injection of oxygenated Ringer's solution into the carotid artery to maintain cerebral circulation during cardiac surgery. Crafoord[3] in 1935 and 1936 showed that the flow of blood to all organs could remain suspended for as long as 20 to 25 minutes in dogs with no sign of subsequent damage provided adequate blood flow to the brain was maintained by creating anastomoses between the carotid and jugular vessels on one side with the corresponding vessels of another dog. O'Shaughnessy[4] in 1939 attempted the perfusion of dog and cat brains with oxygenated hemoglobin during cardiac surgery but few experiments were successful. In 1948 Bjork[5] did some excellent work with the perfusion of dogs brains with blood from mechanical oxygenators; his primary difficulty seemed to be with his mechanical apparatus. Further work along these lines was carried out in 1953 by Battezzati and Taddei.[6]

PROCEDURE

In the initial phase of the experimental work the carotid artery of the donor dog was joined to the distal end of the divided carotid artery of the recipient to provide a flow of oxygenated blood to the recipient's brain. Blood was then returned to the donor by joining the recipient's distal external jugular vein to the donor's proximal external jugular vein. With this circuit supplying oxygenated blood to the recipient's brain the remaining circulation was then interrupted by tying the azygos vein and clamping the cavae. Figure 1 shows the details of this circuit. Thirteen dogs were perfused in this manner, 4 of these having interatrial septal defects made and repaired.

It was then felt that hypothermia might be a useful adjunct to this procedure so the next 21 dogs were subjected to perfusion while in the

*From the Department of Surgery, Stanford University School of Medicine, San Francisco, California. This work was supported by funds obtained from the Life Insurance Medical Research Fund.

hypothermic state In 17 of these dogs cardiotomies were done During this phase of the experimental work occasional changes were made in attempts to equalize the flow of arterial and venous blood Various combinations of pumps and reservoirs were used which seemed only to add to

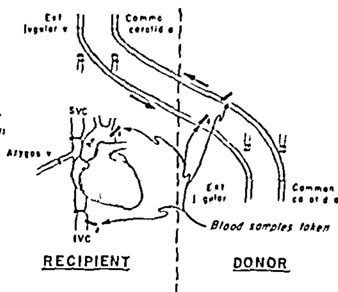


Fig 1 Circulatory connections between dogs in early brain perfusion experiments

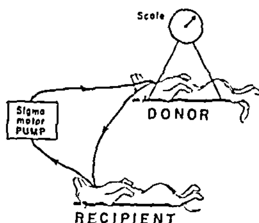


Fig 2 Diagram showing how blood volumes were kept constant in later experiments by keeping donor dog on scale and using pump for return of venous blood

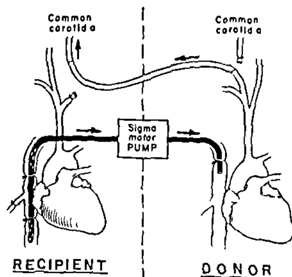


Fig 3 More detailed diagram of arterial and venous connections in later experiments

the complexity of the situation The solution was finally found by placing the donor dog on a scale throughout the perfusion and maintaining a constant weight by altering the speed of a pump used to return the venous blood

Due to the high incidence of ventricular fibrillation in the hypothermic dogs cooling of the recipient was finally abandoned The last 10 dogs were all perfused in the normothermic state with the donor on a scale and the venous blood being returned to the donor with a pump as shown in Figures 2 and 3 Eight of these dogs underwent cardiotomy

In all of these experiments caval occlusion was maintained for varying periods of time ranging from 5 to 45 minutes It seems apparent that 20 minutes is probably the optimum time interval

RESULTS

A total of 52 recipient dogs were used 6 of these not being perfused for various technical reasons There were 2 dogs that died due to technical errors leaving a total of 44 perfusion experiments Twenty two of these dogs survived 24 hours or longer giving an over all survival rate of 50 per cent Of these 22 survivals 16 remained in good health During these 44 experiments there were 8 donor dogs that died 4 of these deaths occurring coincidentally with the recipient deaths Table 2 groups the experimental results according to time of caval occlusion There were 6 dogs that survived the acute stage of the experiments only to have postoperative complications

Table 1 Brain Perfusion Experiments

Total number dogs	52
Dogs not perfused	6
Total dogs perfused	46
Technical errors	2
Total number experiments	44
Total surviving dogs	22
Total healthy dogs	16
Total dead donors	8
Total dead donors with coincident recipient death	4

Table 2 Results of Brain Perfusion Classified According to Time of Caval Occlusion

TIME OF OCCLUSION IN MINUTES	TOTAL NUMBER OF DOGS	TOTAL NUMBER OF DEAD DOGS	TOTAL NUMBER OF LIVING DOGS	TOTAL NUMBER OF HEALTHY DOGS
5 — 10	4	3	1	1
11 — 15	5	[illegible]	3	3
16 — 20	16	7	9	6
21 — 25	7	3	4	2
26 — 30	11	7	4	3
45	1	0	1	1
TOTAL	44	22	22	16

Table 3 Survivals With Residual Damage

LESION	NUMBER DOGS	DEAD DONORS	CARDIAC ARREST OR VENTRICULAR FIBRILLATION
1 CNS damage	5	1	2
2 Infection	1	0	0
TOTAL	6	1	2

Table 4 Operative Course of Dogs Which Became Healthy Survivals

COURSE	NUMBER
1 Uneventful	10
2 Death of donor	3
3 Ventricular fibrillation	2
4 Cardiac arrest	1
TOTAL	16

Table 5 Causes of Death of Recipient Dogs

	NO. OF RECIPIENTS	NO. OF ASSOCIATED DONOR DEATHS
1 Cardiac arrest or ventricular fibrillation	11	1
2 CNS damage	3	0
3 Hemothorax	3	1
4 Unknown	5	2
TOTAL	22	4

One of these eventually died of infection and 5 had signs of cerebral damage. Of the 16 healthy survivals there were 10 having uneventful perfusions; 3 were complicated by the death of the donor and 3 had ventricular fibrillation or cardiac arrest which was converted to normal rhythm. The causes of death in the 22 fatalities are summarized in Table 5. There were 5 dogs in which no abnormality could be found on autopsy but it seems most likely that these dogs died of cerebral anoxia since they were never seen to recover from anesthesia and the heart was in good condition at the time of closure.

Comparison of the results obtained under hypothermia with normothermic perfusions shows a slightly better survival rate under hypothermia but also a higher incidence of ventricular fibrillation. Normothermic dogs had more central nervous system lesions.

DISCUSSION

In attempting brain perfusion in an intact living dog, it should be remembered that the vertebral arteries in the dog are large enough to provide adequate cerebral flow in the presence of bilateral carotid ligation. Therefore during the perfusion these 2 vessels plus the other carotid artery

provide large channels to divert the blood in the circle of Willis away from the brain Undoubtedly, this is the reason there were so many instances of cerebral damage during these experiments

The death of 8 donor dogs casts some doubt on the assumption that this procedure is without danger to the donor It seems likely that some dogs died from bleeding into the recipient during the earlier experiments Even after the use of the scale and pump to prevent this, however, there were 3 donor deaths which is a rather high number to be the result of blood incompatibility

SUMMARY

A method of brain perfusion in experimental open intracardiac surgery has been presented Perfusion of 41 dogs was done with a 50 per cent survival for 21 hours or longer

REFERENCES

1 Warden H E et al Controlled cross circulation for open intracardiac surgery J Thorac Surg 28 331 1954
2 Tuffier T La chirurgie du coeur Rapp Cong Soc Internat Chir (1920) 1921 pp 5 75
3 Crafoord C Congenital coarctation of the aorta and its surgical treatment J Thorac Surg 14 317 1945
4 O Shaughnessy L Future of cardiac surgery Lancet Lond 2 969 1939
5 Bjork V O Brain perfusions in dogs with artificially oxygenated blood Acta chir scand Suppl 96 137 1948
6 Battezzatti M and Taddei C La perfusione della testa isolata in funzione della cardiochirugia a cuore esangue Minerva chir Tor 8 24 925 1953

EXPERIMENTAL CLOSURE OF VENTRICULAR SEPTAL DEFECTS*

JUDAH FOLKMAN

We have developed a closed method for repairing ventricular septal defects This has been accomplished by inserting a polyethylene plate through an intra septal approach and positioning it so as to occlude the defect Other investigators have passed sutures[1] and strips of pericardium[3] through the ventricular septum in attempts to close septal orifices they have all re marked on the safety with which this can be done We have demonstrated that incising the septum to insert a polyethylene plate is neither more dangerous nor more difficult than passing sutures through it

METHOD

Exposure Adult mongrel dogs 18 to 25 kg in weight were anesthetized with nembutal and thoracotomy was performed through the right fourth interspace The costal cartilages of the fourth and fifth ribs were divided near the sternum

Creation of Ventricular Septal Defects High ventricular septal defects were made using a method previously worked out in our laboratory by Dr Elton

*From the Children s Hospital of Boston and the Harvard Medical School Boston Mass

Watkins The pericardial sac is opened A purse string suture is placed around the base of the right auricular appendage and 100 mg pronestyl are injected into the auricle The auricular appendage is pinched off with a clamp and the isolated segment incised a finger is introduced into the auricle and is passed through the tricuspid valve until the ventricular septum can be palpated A special punch is inserted into an incision in the right ventricular wall through a previously placed ventricular purse string Defects of 1 to 2 cm in diameter can be made with this punch When very high defects are made tricuspid regurgitation is generally observed because the septal leaf of the tricuspid valve is usually damaged various degrees of A V block also appear In about one quarter of the animals ventricular fibrillation appeared after punching the defect it was easily abolished with injections of calcium chloride and one or more electric shocks

Closure of the Defect. Two purse string sutures of 3 0 silk are placed just to the right of the anterior descending coronary artery these lie on the anterior surface of the heart in the plane of the ventricular septum (Fig 1) These sutures must circumscribe an area over the septum at least as long as the diameter of the defect and must be placed near enough to the base of the heart that an imaginary line running from this area through the defect will come out in the posterior longitudinal sulcus somewhere inferior to the coronary sinus *Before the sutures are placed* this alignment is easily and quickly determined by the finger which resides inside of the cardiac chamber

An incision within the purse string sutures is made in the plane of the septum and is carried down within the substance of the septum and past the defect This is done with a special 1 cm double-edged blade * guided

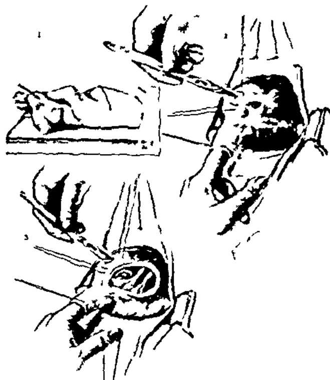


Fig 1 Method of making the septal incision
(1) The right fourth interspace incision used for high septal defects If a fifth interspace incision is used (no cartilages being cut) only mid septal defects can be repaired
(2) The opposing purse strings are placed to the right of the anterior descending coronary artery *The septum is so thick* here that its anterior projection *extends at least 1 cm to the* right of the artery leaving ample *room for the sutures*
(3) The septal incision must be made as wide as the defect and should be stopped at the posterior rim of the defect where only a small nick is made At this point blood which may seep up through the incision is easily controlled by slight tension on the purse strings

* *United Tool* and *Die Co* Cambridge Mass

provide large channels to divert the blood in the circle of Willis away from the brain Undoubtedly, this is the reason there were so many instances of cerebral damage during these experiments

The death of 8 donor dogs casts some doubt on the assumption that this procedure is without danger to the donor It seems likely that some dogs died from bleeding into the recipient during the earlier experiments Even after the use of the scale and pump to prevent this however there were 3 donor deaths which is a rather high number to be the result of blood incompatibility

SUMMARY

A method of brain perfusion in experimental open intracardiac surgery has been presented Perfusion of 11 dogs was done with a 50 per cent survival for 24 hours or longer

REFERENCES

1 Warden H E et al Controlled cross circulation for open intracardiac surgery J Thorac Surg *28* 331 1954
2 Tuffier T La chirurgie du coeur Rapp Cong Soc Internat Chir (1920) 1921 pp 5 75
3 Crafoord C Congenital coarctation of the aorta and its surgical treatment J Thorac Surg *14* 347 1945
4 O Shaughnessy L Future of cardiac surgery Lancet Lond *2* 969 1939
5 Bjork V O Brain perfusions in dogs with artificially oxygenated blood Acta chir scand Suppl *96* 137 1948
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Fig 5 Method of placing the polyethylene plate in the defect (A) The malleable needle is inserted through the septal incision thrust through the posterior part of the septum and pushed out through the back of the heart (B) The polyethylene plate is drawn into the septal incision

proximation of the fifth rib to its cartilage The third death apparently resulted from a complete heart block which appeared immediately after the septal defect had been created

Success of Closure Methods of Healing In the group of 17 surviving dogs 1 was sacrificed immediately after surgery and the remainder were sacrificed while in good health at varying intervals up to 1 year All dogs showed complete closure of the septal defect Three weeks after surgery granulation tissue and some fibrous tissue were beginning to bridge the defect In 1 to 2 months the polyethylene plate was covered over by a fibrous layer Sections for microscopic study of each heart demonstrated that the polyethylene was very inert and caused exceedingly little tissue reaction in the septum (Fig 4)

COMMENTS

The Tricuspid Valve Although the repair of defects required less than 5 minutes of intracardiac manipulations we have found that the finger can be kept through the tricuspid valve for 10 to 15 minutes without any noticeable changes in arterial pressure or electrocardiographic tracings

Disturbances of Conduction Electrocardiographic recordings were taken during surgery and on various successive postoperative days It was interesting that only a few extrasystoles were seen while incising the septum and there was no conduction disturbance once the polyethylene plate was in position

Technical Errors To Avoid During the evolution of this operative technique it was found profitable to remember the following points (a) When the septum is incised the blade must be guided carefully so that it remains *beneath* the endocardium of the septum and does not enter the chamber except at its arrival in the defect It is surprising how accurately the palpating finger can guide the blade (b) When inserting the malleable

by the intracardiac finger The knife is then reversed and the handle used to bluntly widen and deepen the wound into the ventricular septum A semi elliptical polyethylene plate † of appropriate size is selected The plate has been previously prepared with three 3 0 silk sutures with a long



Fig 2 The double edged blade polyethylene plate and malleable needle
(1) Typical plate used to repair a 1 2 cm defect The plate should be large enough so that it completely overlaps the defect and of an elliptical shape with a length at least twice the diameter of the defect With this arrangement the more one pulls on the lead suture the greater is the area of polyethylene that appears in the defect Polyethylene thinner than 035 inch cannot be used since it tends to balloon out of the defect
(2) The special long double edged blade

malleable needle attached to the lead suture (Fig 2) The point of the needle is at the same end as its eye, and this is inserted into the septal incision so that it passes through the septal defect guided by the intracardiac finger and leaves the septum only at the posterior longitudinal sulcus The needle is guided so as not to injure the posterior branch of the right coronary artery An assistant then removes the lead suture from the needle and holds this suture while the needle is withdrawn The 3 point suspension of the plate allows it to be rearranged in the defect until a proper position is obtained as determined by the palpating finger The finger is removed from the heart, and the 3 silks from the polyethylene plate are anchored where they emerge from the surface of the heart The purse strings are now removed the auricular appendage and pericardium are closed the chest is repaired in the usual manner (Fig 3)

RESULTS

After developing the technique this reparative procedure was carried out in a series of 20 dogs using aseptic technique

Mortality Rates Of these 20 dogs, 17 survived the procedure and recovered uneventfully Three deaths occurred on the first postoperative day One was the result of an injury to the posterior descending branch of the right coronary artery made inadvertently by the long malleable needle The second dog died from a punctured lung the result of an inadequate reap

†Polyethylene sheeting 035 inch Forest Products Cambridge Mass

(d) When postoperative systolic murmurs have been present they have always been due to tricuspid regurgitation, and never to patency of the septum

I wish to express my indebtedness to Dr Elton Watkins Jr for his thorough instruction in the method of creating septal defects and to Dr Robert E Gross for his valuable suggestions and kind direction in this study

I would also like to express my gratitude to Mr Norman Budde Miss Cynthia Letteney and Mrs Claire Stiles for their enthusiastic assistance in the surgical procedures

REFERENCES

1 Murray G Closure of defects in cardiac septa Ann Surg 1 8 813 1948
2 Cooley D. Surgical closure of ventricular septal defects Surg Gyn Obst 101 1 3 19
3 Shumacker H B Jr Experimental and clinical observations on the closure of cardiac septal defects Angiology 5 289 19 4

THE ARTIFICIAL HEART LUNG APPARATUS—EXPERIMENTAL CREATION AND REPAIR OF INTERVENTRICULAR SEPTAL DEFECTS*

JACKSON H STUCKEY MELVIN M NEWMAN CLARENCE DENNIS
BERNARD S LEVOWITZ HARRY N ITICOVICI EUGENE J GORAYEB
MARIE KERNAN AND LAVONNE A YOUNG

In July of this year our group working at the State University of New York College of Medicine at New York City reported the development of a pump-oxygenator apparatus and application of it to a patient with intractable cardiac failure[1] The present report is concerned with further application of this apparatus in the creation and closure of interventricular septal defects in experimental animals Gibbon *et al* have described previously creation and repair of interventricular septal defects employing a staged procedure

METHOD

The essentials of the apparatus are indicated in Figure 1 Blood is withdrawn from the venous system of the subject passed through a flow meter and on to slowly revolving stainless steel screen discs which serve as an oxygenator It is collected below the discs passed through a bubble remover consisting of stainless steel sponge coated with Dow Corning anti foam and pumped by a modified Dale Schuster pump back into the arterial system of the subject Catheters are inserted into the superior and inferior venae cavae to siphon venous blood to the oxygenator Sling ligatures are used to occlude the venae cavae adjacent to the heart The apparatus has the ability spontaneously to film additional screening surfaces if the need for them should develop in the course of perfusion The pump has an automatic

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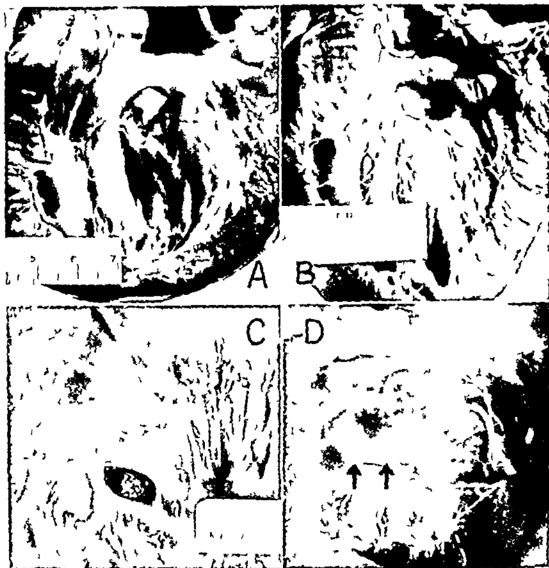


Fig 1 Closure and healing of ventricular septal defects
(A) Typical defect without repair dog sacrificed immediately after production of defect
(B) Complete closure of defect with polyethylene dog sacrificed immediately after surgery
(C) 3 weeks after repair granulation and fibrous tissue covering the polyethylene
(D) 2 months after repair the polyethylene is completely covered by contracted fibrous tissue

needle it should be allowed to slip in easily through the septal slit as far as the septal defect care must be taken to prevent the needle from leaving this prepared channel Obviously if the needle has deviated so as to make another tunnel alongside the septal slit the polyethylene plate will not subsequently enter the septum (c) The malleable needle should remain within the septum at all times This is easily accomplished by placing the palpating finger first superior to the defect then at the rim of the defect and then inferior to the defect as the needle slides by In 1 case where this successive palpation was not done the needle left the defect and entered the right chamber so that the plate was pulled out of the defect and anchored on the right side of the septum When this was discovered the lead suture had to be cut so that the plate could be retrieved and replaced

(d) When postoperative systolic murmurs have been present, they have always been due to tricuspid regurgitation, and never to patency of the septum.

I wish to express my indebtedness to Dr. Elton Watkins, Jr. for his thorough instruction in the method of creating septal defects, and to Dr. Robert E. Gross for his valuable suggestions and kind direction in this study.

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REFERENCES

1. Murray, G.: Closure of defects in cardiac septa. Ann. Surg., *128*:843, 1948.
2. Cooley, D.: Surgical closure of ventricular septal defects. Surg. Gyn. Obst., *101*:[illegible]
3. Shumacker, H. B., Jr.: Experimental and clinical observations on the closure of cardiac septal defects. Angiology, 5:289, 1954.

THE ARTIFICIAL HEART-LUNG APPARATUS—EXPERIMENTAL CREATION AND REPAIR OF INTERVENTRICULAR SEPTAL DEFECTS*

Jackson H. Stuckey, Melvin M. Newman, Clarence Dennis, Bernard S. Levowitz, Harry N. Iticovici, Eugene J. Gorayeb, Marie Kernan and LaVonne A. Young

In July of this year, our group working at the State University of New York College of Medicine at New York City reported the development of a pump oxygenator apparatus and application of it to a patient with intractable cardiac failure.[1] The present report is concerned with further application of this apparatus in the creation and closure of interventricular septal defects in experimental animals. Gibbon *et al* have described previously creation and repair of interventricular septal defects employing a staged procedure.[2]

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The essentials of the apparatus are indicated in Figure 1. Blood is withdrawn from the venous system of the subject, passed through a flow meter and on to slowly revolving stainless steel screen discs which serve as an oxygenator. It is collected below the discs, passed through a bubble remover consisting of stainless steel sponge coated with Dow Corning anti-foam, and pumped by a modified Dale-Schuster pump back into the arterial system of the subject. Catheters are inserted into the superior and inferior venae cavae to siphon venous blood to the oxygenator. Sling ligatures are used to occlude the venae cavae adjacent to the heart. The apparatus has the ability spontaneously to film additional screening surfaces if the need for them should develop in the course of perfusion. The pump has an automatic

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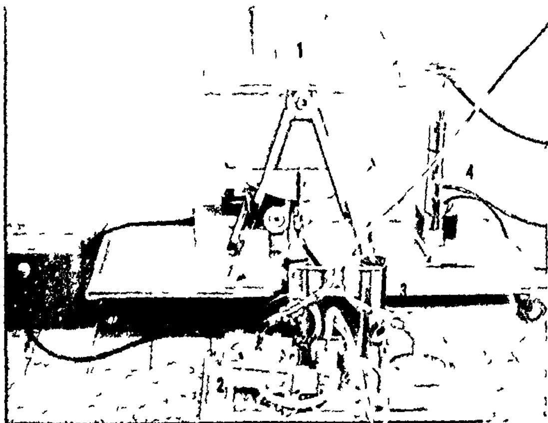


Fig. 1. 1. Oxygenator. 2. Modified Dale-Schuster pump. 3. Bubble trap. 4. Flowmeter.

safety factor in that it injects blood into the arterial system of the subject only if the pump is filled by gravity from the oxygenator prior to each stroke.

The blood utilized to prime the apparatus is prevented from clotting by the addition of 20 mg. of heparin (Connaught Laboratories) for each 500 ml. of freshly drawn arterial blood. The animal to be perfused is given 2.5 mg. of heparin for each kg. of body weight and the titer is confirmed by protamine titration.

While the right ventricle is open, a Foley catheter is in the right atrium (Fig. 2). Distension of the balloon serves to occlude the tricuspid orifice and blood returns from the coronary sinus to the oxygenator through the lumen of the catheter. The coronary sinus return in a 15 kg. dog during perfusion is from 10 to 50 ml. per minute.

The ventriculotomy is made longitudinally over the right ventricular outflow tract and is approximately 6 cm. long. The septal defect, usually 2 cm. long, is made in such a position as to avoid the conduction system insofar as possible. The septal defect is closed with 2 to 4 interrupted silk sutures. The ventriculotomy is closed with interrupted or running silk sutures.

At the end of perfusion a second protamine titration is performed to estimate the amount of protamine needed to return the clotting time to normal. The protamine is given in 5 per cent glucose in water slowly over a period of approximately 15 minutes following which additional protamine

titrations are performed and additional protamine administered as indicated The subject is transfused with 200 to 400 ml of fresh whole blood

RESULTS

Seventeen consecutive animals were operated upon as described These dogs weighed from 8 to 33 kg Two fatalities occurred in these 17 perfusions The first resulted from a pneumothorax during the postoperative hours when one of the thoracostomy tubes accidentally became disconnected from the suction apparatus The second fatality followed inadequate care of the drainage tubes and the animal died with a hemothorax and a bilateral compression atelectasis In neither instance was the pump oxygenator felt to be at fault

Two additional dogs required re exploration for the control of excessive bleeding from the chest during the postoperative period In one the hemorrhage came from the right auricular appendage and in the other from the internal mammary artery In the remaining dogs the average postoperative blood loss into the water seal bottles was 180 ml

COMMENT

Air embolism has not proved to be a problem in this series It has been our experience that pumping systems which reduce the pressure of contained blood far below the atmospheric pressure invite bubble formation both from

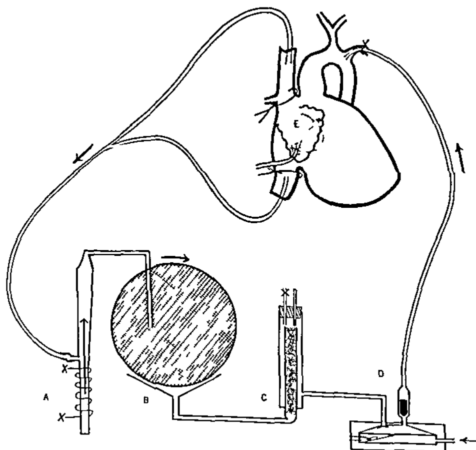


Fig 2 a Flowmeter b Oxygenator c Bubble trap d Modified Dale Schuster pump compressed air enters at arrow e Foley catheter in right atrium

leakage of atmospheric air through defects in the system which are difficult to prevent and from effervescence of gas previously held in solution. Therefore we have placed the oxygenator below the level of the subject and used gravity alone to drain the cavae. For the same reason we have utilized gravity alone to effect filling of the arterial pump from the oxygenator. The self regulatory nature of the modified Dale-Schuster pump is an additional safety factor. In addition, all connections, tubing and pump heads are made of transparent materials; this factor facilitates removal of air bubbles from the system prior to perfusion.

Ventricular fibrillation occurred during cardiotomy in 6 of the 17 dogs. We suspect that both temperature changes and injury to the conduction system may be factors. In every instance, defibrillation was easily accomplished with 1 or 2 electrical discharges of 0.1 second at 1.75 amperes. Defibrillation is undoubtedly facilitated by uninterrupted perfusion of the myocardium with well-oxygenated blood. In those hearts which were defibrillated it was observed that initially a 3:1 atrioventricular block developed which shortly changed to a 2:1 block. Within a few minutes, this was replaced by a normal sinus rhythm.

The ultimate fate of the ventriculotomy wounds has been a source of some concern. These wounds have been observed for periods up to 5 months. No aneurysm has been present upon sacrifice of any animal. A group of dogs is being observed for longer periods after operation.

CONCLUSION

The pump-oxygenator apparatus which this group has previously reported has been utilized in a series of animal experiments in which ventricular septal defects have been created and closed. There appears to have been no mortality attributable to the utilization of the apparatus.

This apparatus has thus far also been utilized on 1 patients. In 2 the patients have been distinctly benefited, 1 with apparent permanent cure. In this overall experience there seems to us to be every cause for optimism and we now seek further appropriate clinical material.

This apparatus has been employed in 4 clinical cases to date. The first was reported by Newman et al. in July of 1955 in which it was possible with a 4 hour perfusion to relieve intractable cardiac failure which had proved resistent to other means of management. The second case was one in which an interventricular septal defect was present. The amount of back bleeding from the aorta was immense and it is the consensus of the group that so much blood was lost from this source that inadequate peripheral blood pressure was maintained permitting air to enter through the relaxed aortic valve leaflets. Air embolism occurred following the addition of sufficient blood to restore the peripheral blood pressure. The third patient was one in which a pulmonic infundibular stenosis with atrial septal defect had been diagnosed. The septal defect was readily closed through an atriotomy incision. The ventricular incision revealed no evidence of pulmonic stenosis. The patient has made an uneventful recovery and appears to have been cured. The fourth case is an additional patient with interatrial septal defect and rather advanced age, 29 years, in whom exploration revealed a totally unsuspected post rheumatic pericarditis with dense adhesions, a persistent atrioventricular canal and in addition rheumatic mitral insufficiency. The defect was closed successfully without development of myocardial irregularities but the patient died of low output right cardiac failure approximately 10 hours after the completion of the procedure.

REFERENCES

1 Newman M M, Stuckey J H Levowitz B S, Young L A, Dennis C, Fries C Gorayeb F J, Zuhdi M, Karlson K, Adler S and Chedman M Complete and partial perfusion of animal and human subjects with the pump-oxygenator Surgery 38 30 1955

2 Gibbon J H, Jr, Miller B J Dobell A R, Engell H C and Voight C B, The closure of interventricular septal defects in dogs during open cardiotomy with the maintenance of the cardio respiratory functions by a pump-oxygenator J Thorac Surg 28 235 1954

CIRCULATORY BYPASS OF THE RIGHT HEART II FURTHER OBSERVATIONS ON VENA CAVAL PULMONARY ARTERY SHUNTS*

José F Patiño William W L Glenn Paul H Guilfoil, Michael Hume and John E Fenn

The complete bypass of the right heart by the caval blood requires the anastomosis of the obstructed superior vena cava to the distal end of one pulmonary artery and the anastomosis of the obstructed inferior vena cava to the distal end of the other pulmonary artery One might expect profound alterations in the movement of the venous (caval) blood through the pulmonary circulation consequent to bypassing the muscular pump of the right heart

The purpose of this investigation is to explore alterations in the hemodynamics following the diversion of at least part of the systemic venous return directly into the pulmonary arterial circulation During the past year we have concentrated most of our efforts in the study of the effects of a shunt made between the superior vena cava obstructed at the level of the right auricle and the distal end of the right pulmonary artery

Fifty nine SVC-RPA anastomoses as shown in Figure 1 A have been performed In 6 other experiments the distal end of the right pulmonary artery has been anastomosed to the side of the inferior vena cava obstructed at the level of the right auricle (Figure 1 B) Three additional attempts have been made to effect a complete bypass of the right heart by the anastomosis of the superior vena cava to the side of the pulmonary artery and by the anastomosis of the inferior vena cava to the distal end of the right pulmonary artery (Figure 1C) In the complete bypass of the heart the main pulmonary artery may or may not be ligated just distal to the pulmonary valves depending on the presence or absence of a septal defect

RESULTS

In the 59 animals where a SVC RPA anastomosis was performed there were 48 experiments where an attempt at survival was made Of these 48

From the Department of Surgery Yale University School of Medicine New Haven Conn Aided by a grant from the United States Public Health Service H 851 C4 C5 The Victoria Fund and the New Haven Heart Association

We are grateful to the Misses Therese Grillo and Leah White Armand Negri and Drs Margaret Albrink and Herbert Harned for their assistance in these experiments

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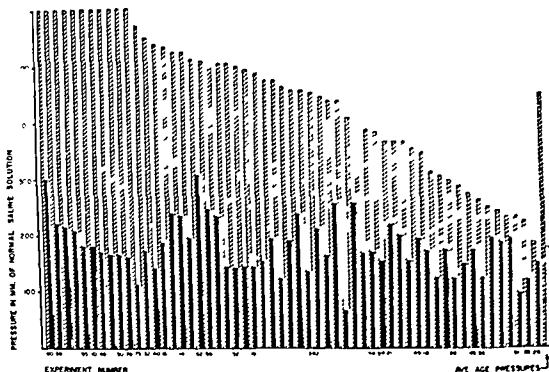


Fig 2

results in 55 experiments where these measurements were made In every experiment the pressure in the obstructed SVC fell markedly when the communication between the SVC and the RPA was opened The average pressure in the occluded SVC prior to opening the anastomosis was 113 mm of saline solution and after the anastomosis was opened the average pressure was 172 mm of saline solution *(2)* One week to 13 months following operation angiocardiography was performed by the injection of diodrast into the superior vena cava[1] An unobstructed flow of dye from the SVC into the RPA was demonstrated in 19 studies on 15 animals In 1 animal a persistent small communication between the SVC and the right auricle was demonstrated and at a subsequent operation this communication was occluded by 2 ligatures and a transfixion suture *(3)* Arterial oxygen saturation studies in 3 acute experiments with the left lung breathing pure nitrogen and the right (shunted) lung breathing room air revealed saturation of the arterial blood of between 30 and 10 per cent In 2 other experiments where the animals were allowed to survive for 9 and 53 days respectively after the creation of a SVC-RPA shunt the animals were again anesthetized and the left main bronchus was clamped for 10 minutes in 1 experiment and for 30 minutes in the other experiment One hundred per cent oxygen was delivered by positive pressure through an endotracheal tube to the right lung The arterial oxygen saturation in both experiments decreased to about 35 per cent during the period of the occlusion of the left bronchus *(4)* Blood flow studies through the shunted right lung were performed in 12 experiments Flow through the right lung was calculated by the Fick principle assuming 96 per cent saturation of the right

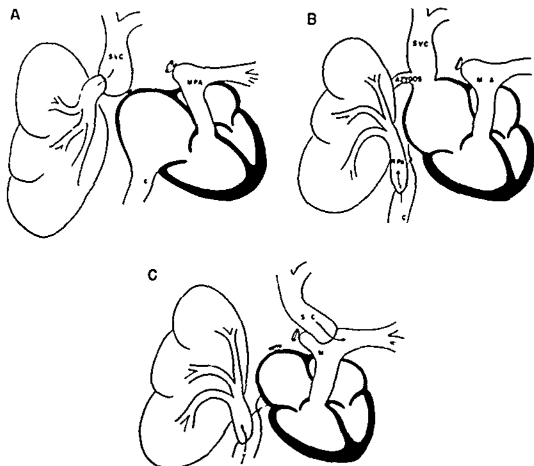


Fig 1 Circulatory bypass of the right heart

animals 15 died within the first 24 hours after operation and 27 died 48 hours to 10 months after operation Six animals are surviving at this time The longest time of survival is 13 months postoperative Of the animals dying within the first 24 hours atelectasis and pneumothorax were the principal causes of death but thrombosis of the SVC RPA anastomosis was not observed

Of the 27 animals dying after 24 hours 5 died during or immediately following angiocardiography and 4 animals died during the performance of blood flow studies Four animals showed thrombosis of the SVC RPA anastomosis at autopsy Significant pleural effusion was found in 20 animals dying after 24 hours and was at least a contributing cause of death in these animals In the majority the effusion was observed to be milky and chemical analysis of the specimens from 4 experiments revealed a high lipid content Three thousand five hundred fifty cc of chylous fluid were found in the pleural cavity of 1 animal at post mortem When the nature of the pleural effusion became evident to us repeated thorocentesis was performed to prevent the accumulation of large amounts of fluid

Several experiments were designed to determine if there was a flow of blood from the superior vena cava into the right lung through the pulmonary artery (1) At the time of operation with the SVC occluded at the right auricle venous pressure was measured before and after the anastomosis of the SVC to the distal end of the RPA was opened Figure 2 shows the

of flow through the right lung some months after the anastomosis. However, when the venous pressure in the SVC was measured several months after the creation of the SVC-RPA shunt, it nearly always showed a decrease from the value obtained immediately postoperative. Whether this finding indicates a decreased pulmonary arteriolar resistance with a possible increased blood flow through the lung or is simply an indication of an expanding collateral venous circulation with a possible decreased blood flow through the lung remains to be determined.

The accumulation of a chylous effusion might have been expected from the experiments of Blalock *et al*[2] who found obstruction of the superior vena cava as a technique for the production of experimental chylothorax in about 50 per cent of their animals. We are attempting to control the chylous effusion by the creation of a wide anastomosis between the SVC and RPA, by prophylactic ligation of the thoracic duct above the diaphragm at the time the SVC-RPA shunt is made and by the repeated aspiration of the effusion should it accumulate.

REFERENCES

1 Glenn W W L and Patino J F Circulatory bypass of the right heart I Preliminary observations on the direct delivery of vena caval blood into the pulmonary arterial circulation Azygos vein pulmonary artery shunt Yale J Biol *27* 3 147 151 1954
2 Blalock A Cunningham R S and Robinson C S Experimental production of chylothorax by occlusion of superior vena cava Ann Surg *104* 359 364 1936

pulmonary venous blood. The venous blood sample was obtained from the superior vena cava. During the flow study the left lung breathed pure nitrogen and the right lung breathed room air.

Flow studies were considered satisfactory in 6 experiments. In 4 of these experiments the flow study was performed immediately after the SVC-RPA shunt was opened with the SVC ligated as it entered the right auricle. Unfortunately, total cardiac output was not determined in these experiments. In 2 other experiments 1 and 3 months after the SVC-RPA anastomosis was made, total cardiac output and blood flow through the right lung were calculated. Table 1 summarizes the values obtained in flow studies done by both techniques (5). Patency of the anastomosis and of the distal pulmonary arterial tree was determined at postmortem by direct inspection and in 4 animals by the injection of the SVC-RPA circuit with vinyl plastic followed by acid digestion of the tissue.

Table 1. S.V.C.-R.P.A. Shunt. Blood Flow Through Right Lung

EXPERIMENT NUMBER	WEIGHT KILO	DURATION OF SHUNT POSTOPERATIVE	CARDIAC OUTPUT	RIGHT LUNG FLOW	
			LITERS PER MINUTE		% OF CARDIAC OUTPUT
37		Immediately		417	
39	12	immediately		598	
40	16	immediately		603	
42	11	immediately		193	
47	10	3 months	2.3	322	13.5
75	26	1 month	2.52	875	34.7

In addition to the above experiments, 6 experiments were completed with anastomosis of the IVC to the RPA (Figure 1 B). Two of these animals survived for long terms. One of these was sacrificed at 53 days and showed a patent anastomosis. The second animal is surviving 10 months following operation. In this second animal an angiocardiogram performed through the IVC 29 days following operation showed a patent anastomosis. A more recent angiocardiogram 10 months after operation failed to demonstrate the passage of dye from the IVC into the RPA.*

In 3 experiments a complete bypass of the right heart has been attempted (Figure 1 C) but none of these animals have survived operations. However, in 2 experiments with an SVC-RPA anastomosis, the IVC has been ligated below the renal veins. Both animals survived until sacrifice several months later.

DISCUSSION

From the data presented there is no doubt that there exists a gradient of pressure from the obstructed superior vena cava to the distal right pulmonary artery. The evidence suggests that at least soon after the anastomosis of the SVC to the RPA, approximately 30 to 40 per cent of the total venous insufficient data at present to determine if there is a change in the quantity

*At the time of sacrifice the anastomosis was found to be widely patent. A vinyl plastic cast was made of the specimen.

was opened and ligatures passed around the left brachial (subclavian) artery and the aorta. A polyethylene catheter was inserted proximally into the right internal mammary artery in order to measure pressure during the period of bypass. A polyethylene catheter was inserted into the right brachial or right carotid artery for subsequent perfusion of the aortic arch. The catheter was connected through a finger pump to a reservoir of oxygenated blood. Another catheter was inserted into the superior vena cava through the azygos vein. The perfusion is shown schematically in Figure 1.

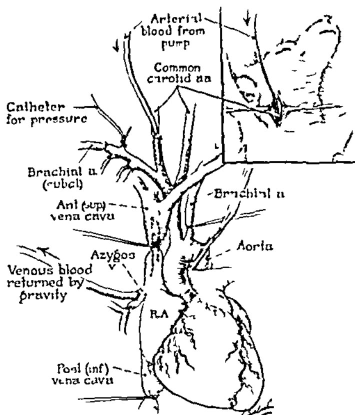


Fig. 1. Diagrammatic Representation of the Perfusion System.

The superior vena cava was then occluded and its blood siphoned into a graduated cylinder. The left brachial artery was temporarily occluded and the right also if it was not used for perfusion. The inferior vena cava was next occluded. The aorta was cross clamped immediately distal to the origin of the left subclavian artery and perfusion begun. An incision 1 to 5 cms. in length was made in the right ventricle and the coronary sinus blood continuously aspirated. The ventricle was then closed, the lungs were inflated, normal circulation restored, and perfusion discontinued. In all animals the right ventricle was open for more than 15 minutes and in the last 13 for 22 to 27 minutes.

The arterial blood used for perfusions was obtained from the femoral arteries of donor dogs. The blood was collected in bottles containing 40 mg. of heparin per liter of blood. No determinations of blood incompatibility were made and frequently pooled blood from 6 or 7 dogs was used. The blood was pumped from a 2 liter graduate after filtration through commercial recipient sets.

In most animals the sum of the blood collected from the superior cava

Surgical Problems in Diseases of the Coronary Arteries and in the Great Vessels

CORONARY AND CAROTID ARTERY PERFUSION DURING TOTAL BYPASS OF THE HEART*

ROBERT A. GAERTNER, JAMES ISAACS, RICHARD DIVER, AND JEROME H. KAY

During the past year in the course of 171 experiments a simplified method of total cardiac bypass has been developed. In the first group of experiments with a dispersion type oxygenator[1] and a finger pump the heart was completely bypassed for 15 to 30 minutes during which time the right ventricle was open. The entire animal was perfused and a systolic pressure of 80 mm Hg was usually maintained. Of 27 dogs perfused all except 1 died within 24 hours. The remaining animal was sacrificed 2 months later. Most of the dogs died immediately after perfusion with a progressive fall in blood pressure which did not improve with transfusion or vasopressors. Those that survived this initial period usually died of hemothorax. Because of these discouraging results it was felt that the survival rate could be improved if the heart and head alone were perfused. In this way the pool of normal blood in the rest of the body would not enter the extracorporeal circuit. The technique and results in 75 consecutive experiments are presented.

METHOD

Dogs weighing 11 to 55 kg were used. Anesthesia was induced with 2½ per cent pentothal and an endotracheal tube was inserted. The animals were maintained with ether and oxygen administered with intermittent positive pressure. The femoral artery and vein were cannulated for pressure measurements. The dogs were cooled in a water and ice bath (3° to 4° C). The first 55 animals were removed from the bath at a rectal temperature of 30° to 32° C and the last 20 at 35° C. During cardiac bypass the temperatures in the first group were 26° to 29° C and in the second 32° to 33° C. Sterile technique was not employed.

A right thoracotomy was performed through the fourth intercostal space. A heavy silk ligature was passed around each vena cava. The right brachial (subclavian) artery was isolated and ligatures were passed around it and all of its branches except the internal mammary. The mediastinal pleura

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ACCURATE DIAGNOSTIC CORONARY ARTERIOGRAPHY IN THE DOG*

JACK A CANNON CHARLES A CLIFFORD GAUTAM DESH AND
WILEY F BARKER

It is our belief that certain cases of arteriosclerotic obliterative coronary artery disease are amenable to treatment by endarterectomy At present no reliable clinical methods are available that permit the precise localization of a coronary artery obstruction Even the localization of an infarct as to its position in the heart anterior versus posterior cannot always be reliably accomplished In addition by presently available methods a diagnosis of coronary artery disease is not always possible until 1 to 2 days after an acute occlusive episode

With the hope of perfecting a reliable method of localizing obstructive lesions in the coronary tree we have experimented with techniques of coronary angiography in the dog The following is a preliminary report of our favorable experience with a practical method

METHOD

Medium to large sized dogs were used The dog was anesthetized with nembutol and the anterior neck region was prepared and draped A 4 cm mid line incision was made beginning just below the inferior border of the

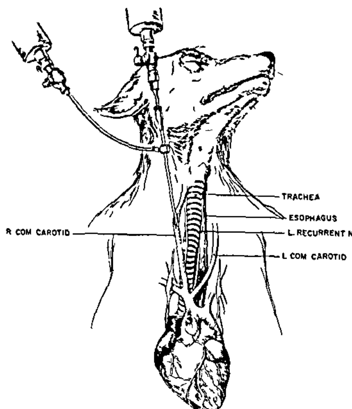


Fig 1 Sketch showing cannula in place in ascending aorta with balloon inflated via side attached syringe Radiopaque dye is injected through end attached syringe

* From the Department of Surgery University of California Medical Center Los Angeles California This work has been supported by Grant Number H 1787 United States Public Health Service

and from the right ventricle was 150 to 250 cc greater than the amount perfused during the period of bypass This deficit was replaced with an intravenous transfusion of citrated blood after restoration of the circulation The animals were given protamine sulfate (1 mg/kg) to neutralize the heparin that remained following perfusion

RESULTS

All but one animal survived the perfusion Twenty seven died within 72 hours postoperatively and 17 were long term survivors All of the animals which died were found at autopsy to have varying degrees of pulmonary congestion In most this was the only apparent cause of death There was no correlation between the duration of perfusion and length of survival

The systolic pressure in the internal mammary artery ranged between 90 and 120 mm Hg during the period of bypass in all animals The amount of blood perfused per minute varied from 150 cc in a 11 kg dog to 282 cc per minute in a 55 kg St Bernard Larger dogs required the perfusion of less blood per kg of body weight to maintain a pressure of 90 to 120 mm Hg in the perfused segment The 11 kg dog was perfused at the rate of 15 cc per kg per minute compared with only 5 cc per kg per minute in the larger St Bernard The aorta was clamped for 17 to 37 minutes in these dogs without evidence of neurological renal hepatic or intestinal damage None had evidence of cerebral dysfunction

DISCUSSION

In our early experiments with carotid and coronary artery perfusion the body temperature was not lowered However with the aorta and both brachial (subclavian) arteries clamped cord damage always occurred with periods of occlusion greater than 10 to 12 minutes Hypothermia was then employed with rectal temperatures of 25 C at the time of bypass Because of the high incidence of ventricular fibrillation at this temperature the animals were perfused at progressively higher temperatures Fibrillation did not occur at 32 to 33° C 17 of 20 dogs perfused at this temperature survived until sacrificed 4 to 6 weeks later

The technique described has certain advantages over other methods for cardiac bypass The equipment is not complex Since only the head and the heart are perfused normal blood pressure can be maintained with a relatively small flow Ventricular fibrillation rarely occurs There is no abnormal postoperative bleeding since a large pool of normal blood does not enter into the perfusion and total heparinization is unnecessary Since cerebral circulation is maintained profound hypothermia is not required to prevent neurological damage

SUMMARY

A technique is described for total cardiac bypass permitting right ventriculotomy for long periods The head and heart are perfused with oxygenated heparinized blood at a body temperature of 32 C Of 20 consecutive animals 17 were long term survivors

REFERENCE

1 Clark L C Jr Hooven F and Gollan F A large capacity all glass dispersion oxygenator and pump Rev Sc Instrum 23 748 753 1952





Fig 2 Fig 3

Fig 2 Normal coronary angiogram Fig 3 Repeat coronary angiogram Same animal as Fig 2 but angiogram taken 1 week after ligatures had been placed at two points on the anterior descending branch of the left coronary at points indicated by arrows Note dilatation of small branch immediately proximal to upper ligature in Fig 3 as compared with Fig 2

The location of the point of artery ligation was confirmed in each case by sacrificing the dog performing a postmortem angiogram on the heart and demonstrating myocardial infarction distal to the point of ligation In no instance was significant secondary clotting noted in the vessel distal to the point of ligation Usually angiography and postmortem dissection demonstrated continued patency of the distal vessel after ligation This observation coincides with that which is demonstrable frequently to a greater or lesser degree in the hearts of patients dying of coronary artery disease

It was apparent from our observations that the point of ligation of the main trunk of the anterior descending branch of the left coronary could always be visualized accurately by this method of angiography Obstructions in smaller branches of this vessel could usually but not always be demonstrated

CONCLUSIONS

Accurate localization of small vessel obstructions in the coronary arterial tree by angiography is feasible

Radiopaque solutions 1 to 10 times the relative maximum volume commonly used in human angiography did not appear to irritate the heart or give other than momentary electrocardiogram changes One death occurred in 51 injections The maximum total volume injected at any one procedure was 36 cc in 3 separate portions

Coronary angiography in dogs with areas of myocardial infarction did not appear to be injurious to the heart or to jeopardize the survival of the dog

It is suggested that a modification of this method could be applied in human coronary obstructive disease with good diagnostic result and with a morbidity no more severe than that experienced in cerebral angiography

Satisfactory films were obtainable without a rapid cassette changing device provided proper attention was paid to timing of the injection and exposure If a multiple exposure method was employed an even greater accuracy could be expected

REFERENCE

1 Agress C M Rosenberg M J Jacobs H I Binder M J Schneiderman A and Clark W G Protracted shock in the closed chest dog following coronary embolization with graded microspheres Am J Physiol *170* 536 549 1952

larynx Dissection was carried down to either common carotid artery which was isolated and controlled with bulldog clamps The animal was then transferred to the x ray table and centered over a 7 x 12 cm cassette A 5 mm longitudinal opening was then made in the vessel The tip of a special double lumen metal cannula (Fig 1)[1]† was inserted into the proximal carotid and from there downward into the aortic arch and ascending aorta until the tip was felt to rest against the aortic valve The cannula was then withdrawn about 1 cm In order to obtain a satisfactory film from this point on careful timing was necessary On signal from the surgeon the assistant inflated the cannula balloon which resulted in temporary almost complete occlusion of the ascending aorta The surgeon then rapidly injected 10 cc of 70 per cent Iodokon® Just before completion of the injection the film was exposed (Non Bucky exposure using 100 ma 60 kv $\frac{1}{10}$ second 36 inches The primary beam was filtered by 1 mm of aluminum) The cassette was removed and the film developed If the film was unsatisfactory a second injection and exposure was made without apparent harm After removal of the cannula the dog was then transferred back to the operating table the carotid artery was repaired using 000000 arterial silk and the wound was closed

In order to test the accuracy of the method each dog was subjected to thoracotomy after normal angiograms had been obtained and a branch of the left anterior descending coronary artery was ligated The wound was closed and after a period of 1 to 7 days a second coronary angiogram was made The dog was then sacrificed and the heart carefully examined by making a postmortem angiogram and searching for gross and microscopic evidence of infarction

RESULTS

In 14 dogs a good to excellent preliminary or normal coronary angiogram was obtained after an average of 2 injections and 2 exposures One dog died in this group a few minutes after a second injection and exposure We were unable to determine an exact cause of death Electrocardiograms obtained before during and after injection of the opaque medium revealed only transient effects in the form of arrhythmias and bizarre tracing changes which lasted a few seconds to 2 minutes No permanent changes were noted

The 13 dogs surviving preliminary angiograms were then subjected to ligation of a distal segment of the anterior descending branch of the left coronary or a large branch of this vessel One of these dogs died a few hours after thoracotomy probably of aspiration of vomitus Four to 7 days later the 12 surviving dogs were subjected to a secondary coronary angiogram Slightly less than an average of 2 injections and exposures were made Localization of the point of ligation in the coronary tree was possible by x ray in 9 out of the 12 dogs In 2 the vessel ligated was very small and was not visualized though good films of most of the coronary tree resulted In another a failure occurred because of faulty technique None of the dogs showed significant ill effects from the repeat angiograms even though in most instances it was necessary to use the remaining carotid artery for passage of the cannula (Figs 2 3)

†Grateful acknowledgement is made to Dr C M Agress who kindly permitted us to use a prototype of the double lumen cannula which he designed and which he used in other experiments on the coronary arterial tree

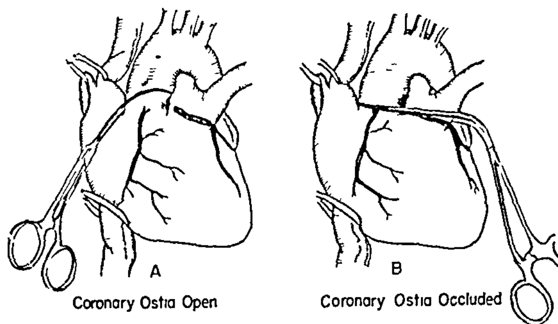


Fig 1 The method for obtaining the 2 types of outflow occlusion is illustrated

the superior and inferior venae cavae and azygos vein and aortic outflow occlusion of the ascending aorta distal to the coronary ostia right ventriculotomies (3 to 4 cm long) and closures were done on 30 dogs 20 with no protection and 10 with 0 35 cc of 1 1000 neostigmine/kg by coronary perfusion (a technique previously described[1]) The duration of inflow occlusion was always 10 minutes In these animals the clamp was in the position shown in A of Figure 1

The experiment was repeated using neostigmine by coronary perfusion in 10 additional dogs except that following the perfusion of neostigmine the clamp was removed after 6 beats and a non crushing clamp was placed through the transverse sinus and positioned as in ' B of Figure 1 to occlude pulmonary artery aorta and coronary ostia Our standard ventriculotomy was then done A similar series of experiments was done in 14 hypothermic dogs using bethanechol chloride (0 5 mg to 0 05 mg /kg) by coronary perfusion before ventriculotomy The coronary ostia and pulmonary artery were open in 8 dogs and occluded in 6 The inflow occlusion in this group was from 10 to 20 minutes in duration

The second experiment was designed to measure the coronary flow during inflow occlusion with the 2 different types of occlusion Cannulation of the coronary sinus was accomplished by passing a suitable catheter with a curved tip down the right external jugular vein Through a right fifth interspace thoracotomy, the tip was inserted into the coronary sinus without opening the heart The distal end of the catheter was connected by a Y tube to another catheter in the left jugular vein A ligature was passed about the coronary sinus to hold the catheter tip just within the sinus ostia Heparin 2 mg /kg was given intravenously every 3 to 4 hours The thoracotomy was closed and the dog cooled Nine dogs were prepared in this manner The coronary sinus flow was measured following inflow occlusion with coronary ostia open (Figure 1A) and occluded (Figure 1B)

THE RELATION OF CORONARY BLOOD FLOW TO PREVENTION OF VENTRICULAR FIBRILLATION IN THE COLD CANINE HEART*

SYLVAN B BAER A VERNON MONTGOMERY EMIL BLAIR AND HENRY SWAN

Previous work from our laboratory[1] has shown that neostigmine by coronary perfusion at least partially protects the cold canine heart from ventricular fibrillation during right ventriculotomy and closure during inflow occlusion for periods up to 10 minutes This protection is thought to be due to the anticholinesterase effect of neostigmine which permits an accumulation of acetylcholine This hypothesis is substantiated by a similar effectiveness of vagal stimulation and acetylcholine by coronary perfusion in preventing fibrillation[1]

However since this work was done the type of aortic outflow occlusion was found in our experiments, to be a critical aspect of the protective effect of neostigmine during ventricular manipulation in hypothermic dogs at 26° C with inflow occlusion Occlusion of the coronary ostia by the outflow occluding clamp (a maneuver useful in cardiac surgery for preventing coronary air embolus during intracardiac surgery) markedly diminished the protective effect of neostigmine We felt this might be related to the decreased coronary blood flow resulting from the occluded coronary ostia Since neostigmine is a cholinesterase inhibitor and exerts its action at least in part[2] by permitting the accumulation of acetylcholine it is suggested that the occlusion of the coronary ostia in some way alters the synthesis of acetylcholine

The altered effectiveness of neostigmine led us to explore the antifibrillatory effects of a stable acetylcholine like drug bethanechol chloride which is unaffected by cholinesterase[3] and led us to measure the coronary flow in the inflow occluded hypothermic dog with and without coronary ostia occlusion

METHOD

Two groups of experiments are presented (1) those concerned with the effect of certain drugs on ventricular fibrillation in hypothermia with the different types of outflow occlusions described in Figure 1 and (2) those concerned with the difference in coronary blood flow resulting from these 2 types of outflow occlusions

Sixty three apparently healthy mongrel dogs weighing 7 to 16 kg were anesthetized with intravenous nembutal (35 mg /kg) Endotracheal tubes and rectal thermocouples were inserted and the dogs were placed in an ice water bath During cooling and throughout the experiments all animals were hyperventilated with 100 per cent oxygen (10 to 60 respirations per minute) At 28 to 29 C the dogs were removed from the ice bath and allowed to stabilize usually 3 to 4° C lower Under sterile technique right fourth interspace thoracotomies were done With umbilical tapes occluding

*From the Departments of Surgery and Physiology University of Colorado Medical School Denver Colorado Aided in part by grant from the United States Public Health Service (H 1559) and in part by a contract with the United States Army (DA 49 007 MD 572)

effective with or without a small coronary flow This difference is compatible we believe with the following explanation

To be effective neostigmine requires acetylcholine synthesis acetylcholine requires ATP for synthesis[4,5] and ATP is supplied mainly by the breakdown of glucose[6] In the aerobic oxidation of 2 moles of pyruvate derived from 1 mole of glucose 30 moles of ATP are formed[6,7] In the anaerobic breakdown of 1 mole of glucose to lactate only 2 moles of ATP are formed[6,7] With the coronary ostia occluded anaerobic synthesis of ATP prevails Therefore acetylcholine synthesis fails[4] and neostigmine is ineffective Bethanechol chloride however is a stable acetylcholine like substance unaffected by cholinesterase[3] and needs no aerobic cycle from a partial coronary flow for synthesis since it is introduced by coronary perfusion

That the above reactions can occur in heart muscle has been shown Adenosinetriphosphate has been used to synthesize acetylcholine in heart muscle slices[5] The enzymes needed for the synthesis of acetylcholine have been isolated from muscle[7,8,9]

CONCLUSIONS

1 The protective action against ventricular fibrillation of neostigmine was greater with a partial coronary flow than with occlusion of coronary flow

2 Bethanechol chloride protection action was independent of coronary flow

3 Because of the high late mortality in the bethanechol chloride experiments the drug is considered dangerous until dose levels and other safety factors have been analyzed

REFERENCES

1 Montgomery A V Prevedel A E and Swan H Prostigmine inhibition in the hypothermic dog Circulation N Y 10 721 727 1954
2 Koelle G B and Gilman A Anticholinesterase drugs Pharm Rev 1 166 216 1949
3 Molitar H A comparative study of the effects of five choline compounds used in therapeutics J Pharm Exp Ther 58 337 360 1936
4 Nachmansohn D and Machado A L The formation of acetylcholine A new enzyme Cholineacetylase J Neurophysiol 6 397-403 1943
5 Bülbring E and Burn J H Action of acetylcholine on rabbit auricles in relation to acetylcholine synthesis J Physiol Lond 108 508 524 1949
6 Ochoa S Efficiency of aerobic phosphorylation in cell free heart extracts J Biol Chem 151 493 505 1943
7 Nachmansohn D Metabolism and function of the nerve cell The Harvey Lecture Series Springfield Ill Chas C Thomas 1953 54 pp 57 59
8 Nachmansohn D and Rothenberg M A Studies on cholinesterase I On the specificity of the enzyme in nerve tissue J Biol Chem 158 653 666 1945
9 Koelle G B The histochemical differentiation of types of cholinesterase and their locations in tissues of the cat J Parm Exp Ther 100 158 179 1950

RESULTS

With inflow occlusion and outflow occlusion of the ascending aorta distal to the coronary ostia right ventriculotomy in our laboratory uniformly causes ventricular fibrillation in unprotected dogs at 25° C However, neostigmine by coronary perfusion after inflow occlusion but before ventriculotomy prevented ventricular fibrillation during the 10 minutes of circulatory occlusion in 8 of 10 dogs, with long term survival of the dogs With the coronary ostia occluded, however neostigmine offered inadequate protection for the entire 10 minutes of ventricular stimulation under these conditions Seven of 10 dogs fibrillated the fibrillation beginning usually about 5 minutes after mechanical stimulation

Bethanecol chloride by coronary perfusion in contrast to neostigmine protected the cold canine inflow occluded heart from ventricular fibrillation for 20 minutes of mechanical stimulation of the ventricle (ventriculotomy) irrespective of occlusion of the coronary ostia and pulmonary artery In the dosages used (0 5 mg to 0 05 mg /kg) and under the conditions of the experiment (prolonged occlusions with brain damage and severe cardiac trauma, creation of inter ventricular defects, etc) late mortality from causes other than fibrillation was high

Using our coronary sinus preparation we found that following inflow occlusion with the coronary ostia open coronary sinus flow derived from the myocardium and pulmonary reservoir though by no means normal is greater and continues longer than with the clamp occluding pulmonary artery and coronary ostia (Figure 2)

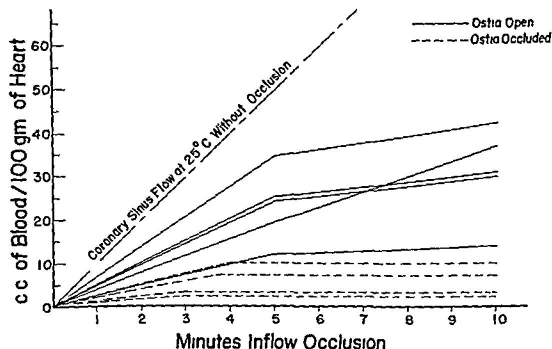


Fig 2 *The coronary sinus flow is shown following inflow occlusion with coronary ostia open and occluded*

DISCUSSION

These experiments seem to indicate that neostigmine is effective as long as there is a partial coronary circulation but that bethanecol chloride is

effective with or without a small coronary flow This difference is compatible we believe with the following explanation

To be effective neostigmine requires acetylcholine synthesis acetylcholine requires ATP for synthesis[4,5] and ATP is supplied mainly by the breakdown of glucose[6] In the aerobic oxidation of *2* moles of pyruvate derived from 1 mole of glucose 30 moles of ATP are formed[6,7] In the anaerobic breakdown of 1 mole of glucose to lactate only *2* moles of ATP are formed[6] With the coronary ostia occluded anaerobic synthesis of ATP prevails Therefore acetylcholine synthesis falls[4] and neostigmine is ineffective Bethanechol chloride however is a stable acetylcholine like substance unaffected by cholinesterase[3] and needs no aerobic cycle from a partial coronary flow for synthesis since it is introduced by coronary perfusion

That the above reactions can occur in heart muscle has been shown Adenosinetriphosphate has been used to synthesize acetylcholine in heart muscle slices[5] The enzymes needed for the synthesis of acetylcholine have been isolated from muscle[7,8]

CONCLUSIONS

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REFERENCES

1 Montgomery A V Prevedel A E and Swan H Prostigmine inhibition in the hypothermic dog Circulation N Y *10* 721 727 1954
2 Koelle G B and Gilman A Anticholinesterase drugs Pharm Rev *1* 166 216 1949
3 Molitar H A comparative study of the effects of five choline compounds used in therapeutics J Pharm Exp Ther *58* 337 360 1936
4 Nachmansohn D and Machado A L The formation of acetylcholine A new enzyme Cholineacetylase J *Neurophysiol* *6* 397-403 1943
5 Bülbring E and Burn J H Action of acetylcholine on rabbit auricles in relation to acetylcholine synthesis J Physiol Lond *108* 508 521 1949
6 Ochoa S Efficiency of aerobic phosphorylation in cell free heart extracts J Biol Chem *151* 493 505 1943
7 Nachmansohn D Metabolism and function of the nerve cell The Harvey Lecture Series Springfield Ill Chas C Thomas 1953 54 pp 57 59
8 Nachmansohn D and Rothenberg M A Studies on cholinesterase I On the specificity of the enzyme in nerve tissue J Biol Chem *158* 653 666 1945
9 Koelle G B The histochemical differentiation of types of cholinesterase and their locations in tissues of the cat J Pharm Exp Ther *100* 158 179 1950

EXPERIMENTAL CHRONIC MYOCARDIAL INSUFFICIENCY PRODUCED BY CORONARY ARTERY EMBOLIZATION*

Gordon Munro, Oscar J. Balchum, J. Cuthbert Owens and Henry Swan

Until the present time no suitable animal with a lesion reasonably similar to arteriosclerotic heart disease in man has been available. Such a preparation is necessary for the evaluation of any surgical technique devised to increase the blood supply of a heart with chronic myocardial insufficiency. The clinical course of patients with arteriosclerotic heart disease is unpredictable and makes evaluation of any revascularization procedure very difficult.

Coronary arteriosclerosis in man is generalized and occurs in the coronary arteries throughout their entire epicardial course. Schlesinger[1] has shown that 2 and occasionally all 3 of the major coronary arteries are occluded in over 50 per cent of the people with angina pectoris and that, in addition, severe narrowing of other ramifications of the epicardial tree occurs.

The techniques used to date in the preparation of dogs with chronic myocardial ischemia have not resulted in pathology similar to that seen in patients with advanced arteriosclerotic heart disease. These methods have involved the opening of the pericardium and the occlusion of a coronary artery. Since the occlusion is limited to a narrow segment of the vessel, bridging collaterals may develop. These techniques also have resulted in the formation of adhesions which may supply blood to the ischemic areas.

In order to produce multiple coronary artery occlusions and to avoid pericardial adhesions, a suitable technique of coronary artery embolization was devised. Many materials have been used for the embolization of the coronary arteries since the procedure was first performed by Panum[2] in 1862. No reports using this technique for the production of chronic coronary insufficiency have, to our knowledge, appeared in the literature other than a brief comment by Agress[3] who first used plastic microspheres in the production of acute coronary shock.

METHOD

Healthy adult mongrel dogs weighing from 15 to 15 pounds were anesthetized with sodium pentobarbitol and maintained on automatic respiration. The aortic arch and its 2 major branches were approached through the left fourth intercostal space and mobilized without disturbing the pericardium. A polyethylene catheter was passed via the carotid artery to the level of the coronary ostia. Styrene divinyl benzene spheres (152 to 608 micra in diameter) kept in suspension with 15 per cent acacia were injected via the catheter. The aortic arch, the left subclavian and brachiocephalic arteries were occluded for 20 seconds while the force of the heart beat ejected the spheres into the coronary vessels. Ten mg of spheres per kg dog body weight was found to be the most acceptable dose through trial and error.

The correlation between exercise tolerance and later pathological findings

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was studied. Exercise tolerance was determined by exercising the dogs on a treadmill. Normal dogs were able to run without distress or dyspnea preoperatively for 30 minutes on a treadmill elevated 15° and traveling at a speed of 6 mph. These dogs were tested at intervals after embolization in a similar fashion. Frequent electrocardiographic tracings were taken using both limb and chest leads both at rest and immediately following exercise. The animals were subsequently sacrificed and the hearts were carefully examined for the localization of spheres and infarcts. The blood vessel pattern was also studied by the injection technique of Schlesinger.[1]

RESULTS

A total of 16 dogs were embolized with 11 surviving. All 11 of these dogs showed multiple myocardial infarctions.

Exercise Tolerance. These dogs could be divided into 2 groups on the basis of their exercise performance after embolization. The first group consisted of 7 dogs showing a definite exercise intolerance which persisted until autopsy 8 to 10 months after embolization when they were capable of running for only 10 to 20 minutes. The second group of 4 dogs were able to run for 30 minutes during the entire post-embolization period of 3 months but were definitely dyspneic before exercise was terminated. In addition 2 control dogs in which embolization was omitted from the operative procedure showed no change in exercise tolerance.

Electrocardiographic Changes indicative of myocardial ischemia and infarction were present in all dogs in both the first and second groups for the first 2 to 3 weeks after embolization. Following this period all tracings were similar to the original electrocardiograms both at rest and after exercise except for minor T wave changes.

Pathology. The dogs which died immediately showed many spheres in the



Fig. 1. Large infarct of lower right ventricle with small area of infarction at base.



Fig 2 (Left) Multiple small areas of infarction of left ventricle (cross section)
Fig 3 (Right) Through and through infarction of wall of right ventricle with several additional small infarcts

epicardial vessels The hearts of the surviving animals all showed multiple myocardial infarctions It was difficult to make a cut in the myocardium without seeing an area of infarction These areas were scattered diffusely throughout the muscle of both the right and left ventricles One dog in the first group developed fresh myocardial infarctions after the exercise period preceding autopsy No pericardial adhesions nor reaction around the spheres were noted

Coronary Artery Visualization Radiopaque material was injected into the coronary arteries according to the Schlesinger technique in 3 dogs of the first group and in all dogs of the second group Multiple coronary occlusions were visualized Well formed collaterals were seen in several of the dogs embolized 6 to 8 months prior to autopsy

DISCUSSION

Exercise intolerance to the point of exhaustion was present in 7 of the 12 dogs (Group I) and persisted until autopsy at 10 months after embolization This might be useful as a future means of estimating the degree of myocardial insufficiency The remaining 4 animals (Group II) showed only dyspnea during the standard exercise test of 30 minutes yet the electrocardiographic and autopsy findings were similar in both groups

It was difficult to account for this difference in exercise tolerance between the 2 groups Investigation concerning a method for the quantitative determination of the amount of total myocardium involved by the infarc

tions is now in progress. However it was estimated that approximately 20 per cent of the heart muscle of these animals consisted of infarcted areas. It was our impression that the hearts of the first group of dogs which had the exercise intolerance were more extensively infarcted. These dogs were smaller in size than the second group which averaged 8 kg. more in weight. It may be that the spheres lodged more proximally in the vessels of these smaller hearts thus producing more myocardial damage with a greater resultant effect on the exercise tolerance.

The occurrence of fresh infarcts after exercise many months after embolization in a dog in the first group strongly suggested that these animals indeed did have myocardial ischemia.

SUMMARY AND CONCLUSIONS

Coronary artery embolization with 10 mg. of plastic microspheres per kg. of dog body weight resulted in multiple coronary occlusions in the epicardial vessels with the production of multiple myocardial infarcts. Chronic myocardial insufficiency as indicated by a reduction in exercise tolerance was present in 7 of the 11 dogs. This experimental preparation therefore is suggested as more closely approximating human chronic myocardial insufficiency than any previously presented.

REFERENCES

1. Schlesinger, M. J.: An injection plus dissection study of coronary artery occlusions and anastomoses. Am. Heart J. *15*: 528, 1938.
2. Panum, P. L.: Experimentelle Beitrage Zur Lehre Von der Embolie. Virchows Arch. *25*: 308, 1862.
3. Agress, C. M., Rosenberg, M. J., Jacobs, H. I., Bender, M. J., Schneiderman, A., and Clark, W. G.: Protracted shock in the closed chest dog following coronary embolization with graded microspheres. Am. J. Physiol. *170*: 536, 1952.

A METHOD OF MYOCARDIAL REVASCULARIZATION USING INTERNAL MAMMARY ARTERY VEIN FISTULAE*

ORMAND C. JULIAN, MARIANO LOPEZ BELIO, HELENIO I. COUTINHO, J. BYRON DAVIS, WILLIAM J. GROVE AND MAX S. SADOVE

The ability of a systemic artery implanted in the myocardium to branch and become collateral to coronary blood flow has been studied by Vineberg.[1] Maintained patency of the implanted artery or its recanalization appear to be basic to its effectiveness in this function as a transplant.

METHOD

In an attempt to attain patency, end to end mammary arteriovenous fistulae were produced and implanted into the myocardium of the left ventricle by two techniques (Fig. 1).

*From the Department of Surgery, University of Illinois College of Medicine, Chicago, Ill. This work was supported by the Ethel and Titus Haffa Fund.

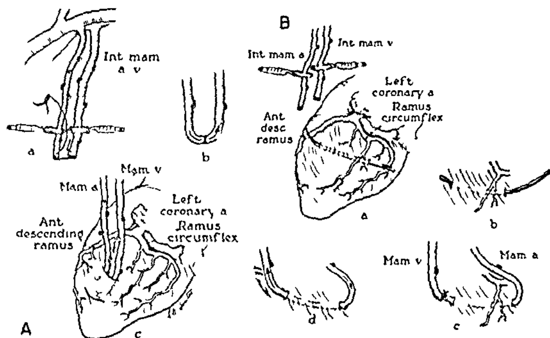


Fig 1 (A) Method of implanting the arteriovenous fistula in Group A (B) Method used in Group B

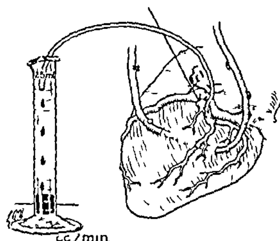


Fig 2 Small polyethylene catheter placed in circumflex artery distal to ligature to measure backflow

A total of 26 preparations in 10 to 15 kg dogs were made Nineteen were made using the technique illustrated in Fig 1A and comprise Group A The 7 prepared as in Fig 1B comprise Group B The fistulae produced no audible bruit although they were all initially active

The animals in Group A were sacrificed at from 11 to 20 weeks Observations were made as to the condition of the arteriovenous fistula and size of the mammary artery Retrograde blood flow from the circumflex artery was measured by catheterizing this vessel distal to a ligature (Fig 2) The survival time after circumflex artery ligation was recorded In two animals arteriographic studies were done in the living state by injection of Thorotrast® into the intact mammary artery (Fig 3) The results of these observations are in Table 1

Table 1 Observations on Animals of Group A

NO	TIME INTERVAL (DAYS)	CONDITION OF FISTULA	SIZE OF ARTERY	RETROGRADE FLOW FROM CIRCUMFLEX (CC/MIN)	SURVIVAL TIME TO FIBRILLATION (MINUTES)
1	119	occluded	small fibrous cord		3.5
2	119	occluded	small fibrous cord		2
3	119	occluded	small fibrous cord	3.8	21
4	105	occluded	small fibrous cord	2.1	22
5	112	occluded	small fibrous cord		2
6	112	occluded	small fibrous cord	0.8	4
7	112	occluded	small fibrous cord		2
8	105	occluded	small fibrous cord		2
9	84	occluded	small fibrous cord		1
10	84	occluded	small fibrous cord	1.8	2.5
11	84	occluded	small fibrous cord	2	3
12	77	occluded	small fibrous cord	2	2
13	77	occluded	small fibrous cord		1.5
14	84	occluded	small fibrous cord	4.8	270
15	210	widely patent	3 mm	not done	not done
16	112	widely patent	4 mm		
17	210	occluded	small cord		1
18	210	occluded	small cord		
19	84	occluded	small cord	2.4	14

Thorotrast injection done for arteriography (Fig 3)

Table 2 Observations on Animals of Group B

NO	TIME INTERVAL (DAYS)	CONDITION OF FISTULA	SIZE OF ARTERY	RETROGRADE FLOW FROM CIRCUMFLEX (CC/MIN)	SURVIVAL TIME TO FIBRILLATION (MINUTES)
20	16	widely open	4 mm	11-9	over 60
21	15	widely open	4 mm	6-8	over 60
22	19	occluded	2 mm	not taken	3
23	22	questionable patency	4 mm	12.5-12.8	over 90
24	16	occluded	2 mm		3
25	22	occluded	2 mm	3.5	320
26	26	occluded	2 mm	1.4-1.2	55

Table 3 Reaction of Animals of Group B Having Open Fistulae to Ligation of Mammary Artery with Circumflex Artery Occluded

NO	RETROGRADE FLOW CIRCUMFLEX ARTERY LIGATED MAMMARY OPEN	RETROGRADE FLOW CIRCUMFLEX LIGATED MAMMARY LIGATED	TIME TO VENTRICULAR FIBRILLATION AFTER MAMMARY LIGATION
20	9 11 cc/min observed for 60 min	4 02 cc/min observed for 11 min	11 min
21	6 8 cc/min observed for 20 min	1.5 1 cc/min observed for 7 min	7 min
23	12 6 cc/min observed for 21 min	1 6 cc/min observed for 30 min	36 min

Arterial lumen not entirely open contained thrombus



A *In vivo* arteriogram in Group A dog number 15 Thorotrast injected into mammary artery

Fig 3



B *In vivo* arteriogram in dog number 16 Group A

The animals of Group B were sacrificed 15 to 26 days following preparation of the fistula The same observations were made as in Group A These are recorded in Table 2 In 2 animals of Group B in which the fistula was widely patent and in one in which it was patent but small additional observations of back flow volume were made with the mammary artery obstructed These data are shown in Table 3

DISCUSSION

In only 5 of the 26 preparations did the fistula remain open and active Two of these were utilized to produce the *in vivo* arteriograms presented These show marked opacification of the general body area of the cardiac shadow These suggest but do not alone demonstrate vascular supply to the myocardium as distinct from adjacent structures

The remaining 3 animals having patent fistulae show a distinctly greater retrograde flow from the ligated circumflex artery than do those having thrombosed fistulae

When the mammary artery in these 3 animals was occluded during measurement of retrograde circumflex flow the rate of flow immediately decreased and fibrillation ensued

Conclusions

1. End-to-end mammary arteriovenous fistulae implanted in the myocardium closed in 21 of 26 animals in this series.
2. Where patency was maintained, the circumflex artery backflow data presented indicates some communication between implanted artery and coronary system.
3. The increase in circumflex artery backflow ceased when the mammary artery was ligated.
4. The single element of mammary artery implantation inherent in the dogs not maintaining their fistulae did not increase circumflex backflow. However, the pre-existing myocardial ischemia used by Vineberg was absent.

References

1. Vineberg, A. M.: Development of anastomoses between coronary vessels and transplanted internal mammary artery. Canad. M. Ass. J. 55:117-119, 1946.

MYOCARDIAL REVASCULARIZATION: A COMPARISON BETWEEN INTERNAL MAMMARY AND SUBCLAVIAN ARTERY IMPLANTATION IN THE DOG*

Maurice C. Fuquay, Lewis S. Carey, Elmer V. Dahl, John W. Kirklin and John H. Grindlay

In view of the fact that anastomotic channels develop between an implanted internal mammary artery and existing arterial vessels in the normal heart in the dog, as has been proved experimentally,[1] we decided to compare histologically and functionally the results of internal mammary implantation with those of a procedure in which the left subclavian artery was implanted. We thought that the larger vessel might assure a greater volume of blood flow, might be less likely to become obliterated as a result of intimal proliferation, and might offer better opportunity for the development of collateral channels.

Internal mammary implantation was performed by dissecting the artery free of the chest wall from the third to the sixth intercostal space. The artery was divided at the level of the seventh rib and freed up. Then the proximal portion with 2 to 4 bleeding branches was drawn into a previously made tunnel in the wall of the left ventricle. Subclavian artery implantation was performed similarly by dividing the vessel distal to its 4 branches and drawing the free portion of the artery, with its branches bleeding freely, into a tunnel in the left ventricle. In both procedures the distal end of the implanted vessel was ligated, drawn completely through the tunnel and attached to the epicardium by a cotton stay suture as shown in Fig. 1. The 2 procedures were compared from the standpoint of (1) protection against death or infarction following ligation of the

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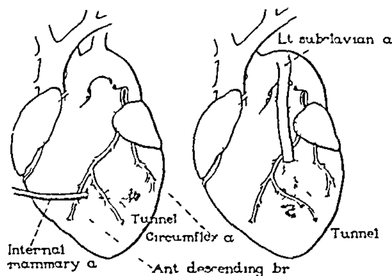


Fig 1 Implantation of (a) the internal mammary artery and (b) the subclavian artery

interior descending branch of the left coronary artery (2) demonstrability of anastomosing branches (3) degree of intimal proliferation of the implanted vessel and (4) the volume of blood flow to the heart through the implanted vessel

The internal mammary procedure was performed on a series of 22 mongrel dogs and the subclavian procedure on a series of 12 dogs A control procedure was performed on 9 dogs In the last group internal mammary implantation was followed immediately by ligation and division of the artery *near the site of entry into the myocardium thus isolating the* implanted vessel from the arterial blood stream In all 3 groups the implantation procedure was followed in 7 to 11 weeks by ligation and division of the anterior descending branch of the left coronary artery near its origin In the internal mammary group a total of 12 dogs (54 5 per cent) survived ligation This compares with 6 survivors (50 per cent) in the subclavian group In the control group there was one survivor (11 1 per cent) All 12 survivors with the internal mammary implant showed microscopic evidence of anterior infarction Of the 6 survivors in the subclavian group 1 was used for the purpose of making an acetate cast of the coronary system and 1 animal is alive awaiting further study The remaining 4 animals were killed Two of these showed no evidence of infarction while the remaining 2 had anterior infarcts In the 1 survivor of the control group there was an anterior infarct

The animals were anesthetized and killed 6 to 24 weeks after ligation of the anterior descending branch of the left coronary artery and the specimens from these animals plus those from animals that died immediately after ligation were injected with a mixture of colloidal suspension of thorium dioxide (thorotrast) and barium for roentgen ray study The injection medium was introduced through the implanted vessel provided it was patent In the internal mammary group there was sufficient patency of the implant to allow filling of the left coronary system in 11 specimens (50 per cent) In the group that survived ligation of the anterior descending artery 8 of 12 vessels (66 7 per cent) were patent while the remaining 4 (33 3 per cent) were not In the group of 10 animals that did not survive ligation of the anterior descending artery patency of the

implant could be demonstrated roentgenographically in 3 (30 per cent) In the subclavian group all implants were patent, including both those from survivors and those from nonsurvivors and there was filling of the coronary system upon injection of the contrast medium In the internal mammary group it was possible to demonstrate definite collateral branches in 1 of 22 specimens (18 2 per cent) In the subclavian group there was definite x ray evidence of small collateral vessels in 8 of 11 specimens studied (72 7 per cent) In all those animals from the subclavian group that had survived ligation of the anterior descending artery there were collateral branches In the control group patency of the implanted vessel or anastomotic channels could not be demonstrated in any of the specimens

In each group the coronary system of representative specimens was injected with vinyl acetate to demonstrate the anastomotic branches connecting the implant and the coronary system

Representative sections of the implanted artery from 10 of the 12 dogs of the subclavian group and from 17 of the 22 dogs of the internal mammary group were examined histologically In each there was increased intimal connective tissue sometimes eccentric composed of collagenous and elastic tissue In the subclavian group the intimal tissue was limited to a narrow rim in 2 dogs it obliterated slightly more than 25 per cent of the original arterial lumen in 3 dogs and it obliterated more than 50 per cent of the lumen in 4 dogs The connective tissue in these 4 was in addition partially vascularized The implanted artery in the tenth dog was partially collapsed and contained a recanalized thrombus

All the transplanted arteries in the internal mammary group presented intimal proliferative changes that were qualitatively similar to but generally more severe than those seen in the subclavian group (Figs 2 and 3) More than 75 per cent of the original lumen had been obliterated by

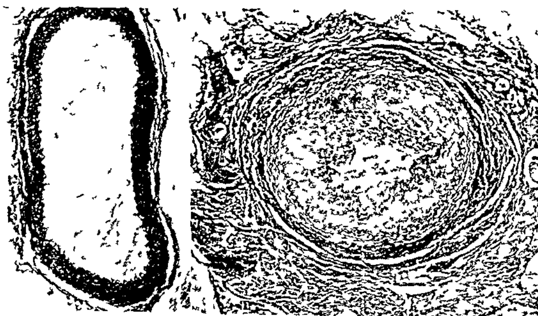


Fig 2 (Left) Normal internal mammary artery with blood in lumen (Verhoeff's elastic tissue stain counterstained with van Gieson connective tissue stain referred to hereafter as ELVG x40) (Right) Internal mammary artery in myocardium 20½ weeks after it had been implanted there More than 75 per cent of the original lumen has been obliterated by intimal connective tissue (ELVG x40)

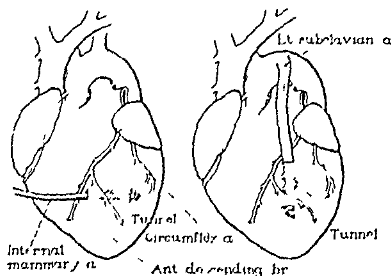


Fig 1 Implantation of (a) the internal mammary artery and (b) the subclavian artery

anterior descending branch of the left coronary artery, (2) demonstrability of anastomosing branches (3) degree of intimal proliferation of the implanted vessel and (4) the volume of blood flow to the heart through the implanted vessel.

The internal mammary procedure was performed on a series of 22 mongrel dogs, and the subclavian procedure on a series of 12 dogs. A control procedure was performed on 9 dogs. In the last group, internal mammary implantation was followed immediately by ligation and division of the artery near the site of entry into the myocardium, thus isolating the implanted vessel from the arterial blood stream. In all 3 groups the implantation procedure was followed in 7 to 11 weeks by ligation and division of the anterior descending branch of the left coronary artery near its origin. In the internal mammary group a total of 12 dogs (54.5 per cent) survived ligation. This compares with 6 survivors (50 per cent) in the subclavian group. In the control group there was one survivor (11.1 per cent). All 12 survivors with the internal mammary implant showed microscopic evidence of anterior infarction. Of the 6 survivors in the subclavian group, 1 was used for the purpose of making an acetate cast of the coronary system and 1 animal is alive awaiting further study. The remaining 4 animals were killed. Two of these showed no evidence of infarction while the remaining 2 had anterior infarcts. In the 1 survivor of the control group there was an anterior infarct.

The animals were anesthetized and killed 6 to 21 weeks after ligation of the anterior descending branch of the left coronary artery and the specimens from these animals plus those from animals that died immediately after ligation were injected with a mixture of colloidal suspension of thorium dioxide (thorotrast) and barium for roentgen ray study. The injection medium was introduced through the implanted vessel provided it was patent. In the internal mammary group there was sufficient patency of the implant to allow filling of the left coronary system in 11 specimens (50 per cent). In the group that survived ligation of the anterior descending artery, 8 of 12 vessels (66.7 per cent) were patent while the remaining 4 (33.3 per cent) were not. In the group of 10 animals that did not survive ligation of the anterior descending artery patency of the

mammary transplants were found only distal to the point of dissection of the vessel from the chest wall, and again the severity of the changes usually increased distally. The severity of the intimal changes in both groups seemed not to depend directly on the length of time the transplanted artery had been in place within limits of the experiments.

From each group animals that survived ligation of the anterior descending branch were heparinized and studied in collaboration with Dr. Hiram L. Essex to determine the volume of blood per minute that reached the heart through the implant. A continuous bubble type flowmeter was engaged into the implant in its free portion just proximal to the heart. In a series of 3 dogs from the internal mammary group there was 1 specimen in which no flow could be demonstrated. This implant was occluded and neither forward nor retrograde flow from the cut vessel was apparent. In the other 2 dogs in this group definite forward and retrograde flow was found, but in each case the flow toward the heart was less than 1 cc. per minute as measured by the flowmeter. In a series of 4 dogs from the subclavian group the flow per minute was respectively less than 1 cc., 7.0 cc., 15.2 cc. and 13.5 cc., and definite forward and retrograde flow was demonstrated in each.

From the foregoing it is evident that implantation of the internal mammary or the subclavian artery into the myocardium affords some protection against death when the anterior descending artery is later ligated. There is no evident difference between the two procedures in the protection afforded against death, although subclavian implantation appears to offer greater protection against infarction. The larger subclavian vessel was less vulnerable to occlusion by intimal proliferation and appeared to afford better opportunity for the development of collateral channels than did the smaller internal mammary vessel. The studies of blood flow performed in each group make it apparent that subclavian implantation might allow a greater volume of blood to flow to the heart.

REFERENCES

1. Vineberg, A. M.: Development of an anastomosis between the coronary vessels and a transplanted internal mammary artery. Canad. M. Ass. J., 55: 117-19, 1946.

MYOCARDIAL REVASCULARIZATION IN THE DOG: EFFECT OF CREATION OF A TEMPORARY FISTULA BETWEEN AN IMPLANTED ARTERY AND THE LEFT ATRIUM*

LEWIS S. CAREY, MAURICE C. FUQUAY, ELMER V. DAHL,
JOHN H. GRINDLAY AND JOHN W. KIRKLIN

Experimental implantation of the internal mammary artery into the myocardium has been shown to result in the development of anastomotic connections between the implant and existing coronary circulation. Significant anastomoses do not occur in every instance and obliterative changes in the wall of the implanted artery are common.[1,2,3,4,5]

A series of experiments on 3 groups of dogs was carried out. In the first group of 22 dogs the internal mammary artery was implanted into the myocardium in a manner similar to that described by Vineberg. In the second group of 9 dogs the same procedure was carried out but immediately after implantation the internal mammary artery was doubly ligated and divided proximal to the site of entry into the myocardium. This second group was a control group. In the third group of 9 animals a temporary communication or fistula was created between the internal mammary artery and the left atrium.

All the dogs were subjected to high ligation of the anterior descending branch of the left coronary artery at a later date to determine the degree of protection afforded by each type of procedure. This paper deals mainly with the description and evaluation of the third group.

PROCEDURE

In the 9 dogs in this group the left internal mammary artery with a segment of the left subclavian artery attached was dissected free from the chest wall down to the seventh intercostal space. Many small elliptical openings were cut in the arterial wall and the artery with several open intercostal arteries was drawn into a superficial myocardial tunnel approximately 5 cm in length which closely paralleled the anterior descending branch of the left coronary artery. The segment of the left subclavian artery attached to the implanted mammary artery was anastomosed to the left atrium. Arterial flow supplied by the lower intercostal, epigastric and diaphragmatic branches of the internal mammary artery, was thus shunted into the left atrium (Fig. 1). Additional procedures will be described with the results.

RESULTS

From 5½ to 9 weeks later the atrial end of the internal mammary artery was doubly clamped and divided between the clamps. Blood flow on temporary release of the superior clamp and then the inferior clamp indicated patency of the atrial stoma and the myocardial implant respectively. Flow occurred at both sites in 8 of 9 dogs. An additional procedure was carried out in 5 of the dogs. The implant was mechanically occluded just

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Fig 1 Operative procedure

proximal to its entrance into the myocardial tunnel The inferior clamp was again removed Flow from the implant was demonstrated in all 5 dogs and ranged from 12 to 21 cc per minute Blood pressures were recorded from the implant in 2 of these animals and values of 91 and 108 mm of mercury were obtained These observations indicate that anastomotic channels of an arterial nature had formed between the implant and the coronary vessels since with occlusion of the implant the blood flow could only come by way of anastomotic connections with the coronary circulation

Ligatures were then placed at the sites of the 2 clamps and the atrial fistula was permanently closed Immediately following this, the anterior descending branch was slowly occluded at its origin and then doubly ligated and divided The septal branch was identified in most cases and left intact Six of the dogs (67 per cent) survived the procedure and 3 died This should be compared to a survival rate of 51 5 per cent in the first group in which the internal mammary artery was implanted into the myocardium and 11 per cent in the control group Electrocardiograms recorded from the surface of the myocardium of 2 of the dogs during the period of gradual occlusion of the anterior descending branch of the left coronary artery were interpreted as showing ischemic changes

Some weeks later operations were again carried out on the 6 surviving dogs and the implant was completely occluded just proximal to its entry into the myocardium for 1 hour This appeared to have no effect on the rate or rhythm of the heart Obviously the systemic connection of the implant at least at this stage was not necessary for the sustenance of cardiac function

An attempt was made in the course of the operation to measure the flow through the implant but unfortunately, because of technical difficulties only meager data were obtained However there was definite flow through the implant and this flow was toward the heart

PATHOLOGIC STUDIES

Following the death of a dog the heart was removed, the implant was cannulated and dyed saline solution was injected through the cannula In all cases the dyed saline solution could be seen flowing through the superficial branches of the left coronary artery and could be recovered from the mouth of the left coronary artery Likewise injection of a mixture of thorium dioxide (thorotrast) and barium through the implant gave roentgenographic evidence of filling of the coronary vessels in all instances In 2 of the hearts the anastomotic channels were large enough so that they were visible on the roentgenogram In contrast an anastomotic connection was shown by the injection of dyed saline solution in only 50 per cent of the first group of animals in which the internal mammary artery was implanted

All 6 dogs in the fistula group surviving ligation of the anterior descending branch of the left coronary artery showed gross and microscopic evidence of healed infarction The infarcts were graded 1 to 3 on microscopic examination according to the depth of involvement of the anterior wall grade 1 signified a subendocardial infarct grade 2 an infarct involving approximately 50 per cent of the thickness of the myocardium and grade 3 a transmural infarct Five of the dogs (83 per cent) showed infarction of grade 1 The remaining dog suffered from infarction of grade 3

Infarction also was present in all the animals in the first group in which the internal mammary artery was implanted into the myocardium It was of grade 1 in 33 per cent grade 2 in 58 per cent and grade 3 in 9 per cent

Sections of various parts of the transplanted artery from each of the 9 dogs with an arterio atrial communication from 8 of the 9 control dogs and from 17 of the 22 dogs in which the internal mammary artery was implanted into the myocardium were examined histologically More than 90 per cent of the original lumen in each of the transplanted arteries in the control dogs was occupied by proliferated intimal connective tissue, usually but not always nonvascular in nature

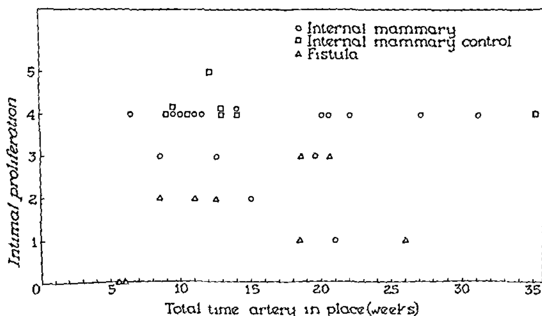


Fig 2 Scatter graph Degree (grade 1 to 5) of obliterative changes in implanted vessels

Increased intimal collagenous and elastic tissue was found in the transplanted artery of each of the dogs in which the internal mammary artery was implanted into the myocardium. This was usually severe as shown in Figure 2 in which the intimal proliferative changes are shown graded on the basis of luminal obliteration.

Similar but generally less severe intimal changes were found in the arteries from 7 of the 9 dogs with arterio-atrial fistulas. No increased intimal connective tissue was apparent in 2 vessels which had been transplanted 5½ and 6 weeks previously. Only 2 of the other 7 vessels showed slightly more than 50 per cent of the original lumen occupied by proliferated intima. Two of the arteries were surrounded in the myocardial tunnel by dilated thin-walled vascular spaces which extended through the surrounding scar into the adjacent myocardium. These vascular channels connected directly to the lumen of the implanted artery at the sites of abrupt defects in their walls, apparently the holes provided prior to implantation. These were the 2 arteries in which the intima remained free of proliferative changes (Fig. 3). No similar vascular channels were found in the scar tissue surrounding the transplanted artery in any of the other experimental groups.

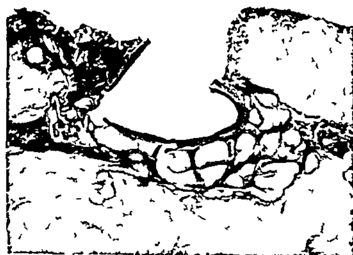


FIG. 3. A portion of the roof of the vessel has been removed. Thin-walled vascular channels filled with injection media surround the implanted vessel. The abrupt defect in the wall should be noted. (Verhoeff elastic tissue stain counterstained with van Gieson connective tissue stain ×12.)

The degree of intimal proliferation in the control group and in the group in which the internal mammary artery was implanted into the myocardium could not be related to the length of time the artery had been in place within the limits of the experiment. As shown in Figure 2 the intimal changes in the group with the arterio-atrial fistula seemed related to duration of the experiment but with individual exceptions.

SUMMARY

Creation of a temporary fistula or communication between the implanted internal mammary artery and the left atrium will produce collateral anastomotic channels of an arterial nature in a normal nonischemic myocardium.

Evidence suggests that this procedure confers a certain degree of protection against death from fibrillation or major infarction following high ligation of the anterior descending branch of the left coronary artery.

REFERENCES

1 Vineberg A M Development of an anastomosis between the coronary vessels and a transplanted internal mammary artery Canad M Ass J *55* 117 119 1946

2 Vineberg A M and Jewett Beverly L Development of an anastomosis between the coronary vessels and a transplanted internal mammary artery Canad M Ass J *56* 609 614 1947

3 Vineberg A M Development of anastomosis between coronary vessels and a transplanted internal mammary artery J Thorac Surg *18* 839 850 1949

4 Glenn Frank and Beal J M The fate of an artery implanted into the myocardium Surgery *27* 841 847 1950

5 Vineberg A M Munro D D Cohen H and Buller W Four years clinical experience with internal mammary artery implantation in the treatment of human coronary artery insufficiency including additional experimental studies J Thorac Surg *29* 1 36 1955

EXPERIMENTAL MYOCARDIAL INFARCTION PRODUCED BY LOCAL BETA RADIATION*

PETER V MOULDER ROBERT W HARRISON WILLIAM E ADAMS AND PAUL V HARPER

The surgery of coronary artery disease has a short history [1] and has had only a few faithful workers There have been 2 formidable problems first the experimental and clinical evaluation of the procedure employed and second the selection of patients for this surgical procedure The second problem will remain unanswered until the first is firmly established All of the operations studied and discussed for coronary artery diseases have as their intention the induction or production of extracoronary cardiac vascular communications It has been anticipated that the additional blood supply would protect against infarct formation when a major channel was occluded that it would afford enough blood to the infarcted area to prevent it from becoming a trigger zone for the development of ventricular fibrillation and that it would afford sustenance for the myocardium while intercoronary anastamoses were enlarging [1, 2]

Experimental evaluation of protective effects has been (1) Decrease in mortality from acute ligation of a major coronary artery (anterior descending branch of the left coronary artery) [3] (2) Decrease in the size of the infarct formed after acute ligation [3] (3) Demonstration of retrograde flow in an artery after its division (circumflex branch of left coronary artery) [4] (4) Injection dissection demonstration of extracoronary myocardial blood supply

This study was undertaken to find means for producing standard experimental subjects for evaluation of coronary operations It was felt that a method for inducing coronary occlusion in the unanesthetized intact animal was needed and that the control should be an infarct without major coronary vascular occlusion Initial studies have shown that thrombosis of

*From the Department of Surgery and the Argonne Cancer Research Hospital of The University of Chicago This study was aided by grants from the Damon Runyon Memorial Fund and the Douglas Smith Foundation for Medical Research of The University of Chicago

the anterior descending branch of the left coronary artery can be produced by the implantation of radioactive yttrium pellets along the side of the vessel Furthermore pellets implanted in the myocardium will produce small but relatively standard areas of necrosis and subsequent fibrosis

METHOD

The production of the radioyttrium pellets has been described and the irradiation data delineated [5]

A lead protected needle is loaded under water placing the pellet in the needle under the lead hood After the needle is introduced into the myocardium or pericoronary fat the obturator is rapidly introduced forcing the pellet into the tissue A pack of wet gauze placed over this affords complete protection while preparing the next pellet

Three groups of dogs were studied (1) Implantation of single pellets in a localized site to demonstrate the size of the destroyed myocardium (Note that multiple separate pellets were introduced into the same heart at one and/or subsequent operations) (2) Implantation of a group of pellets in a sufficiently localized area to produce large (coalescent) sites of destruction (3) Implantation of pellets alongside the anterior descending branch of the left coronary artery with the intention of producing thrombosis of a short segment of the artery and possibly distal infarction

Preoperative and postoperative serial electrocardiograms were obtained in the coronary and coalescent site implantation animals

RESULTS

The experimental data are demonstrated in the accompanying tables Table 1 shows the nature and size of the local destruction of the myocardium produced by the implantation of the single pellet (A) and multiple pellets (B) Table 2 presents the electrocardiographic and postmortem data on the pericoronary implantation animals

Table 1 Effects of Implantation of Yttrium[90] Pellets Into Left Ventricular Myocardium

A SINGLE SITES

DOG NO	NO OF YTTRIUM[90] PELLETS	PELLET STRENGTH MILLICURIES	TIME (S SACRIFICED) (D DIED)	MAJOR DIAMETER OF SINGLE INFARCT MILLIMETERS
265	2	1 0	S 14 days	13 14
292	1	1 0	S 18 days	14
951	2	1 0	S 20 days	12
670	2	1 0	S 21 days	11
667	2	1 0	S 32 days	10 11 5
63	4	1 5	S 5 mos	6 8
	4	1 0	4 mos	
40	4	1.5	S 13 mos	6 8
	5	1 0	12 mos	
107	2	1 0 (Rt Ventricle)	D 1 day	Radioactivity too high for early examination Late postmortem data no good
222	2	1 0	D 2 days	

B. COALESCENT GROUPS

DOG NO.	NO. OF YTTRIUM^{90} PELLETS	PELLET STRENGTH MILLICURIES	TIME (S SACRIFICED) (D DIED)	ECG	SIZE OF INFARCT MILLIMETERS
292	7	1.0	S 18 days	Early Infarction Pattern	5 Sites
	2				17 x 12 x 11
	1				25 x 10 x 10
121	6	1.0	S 5 mos	Minimal Chest Lead Changes	21 x 17 x 2 to 6
653	5	0.5	S 4 mos	No Changes	16 x 11 x 5
613	4	1.0	S 4 mos	Minimal Chest Lead Changes	Inaccurate Data

Table 2. Effects of Implantation of Yttrium^{90} Pellets Along Anterior Descending Left Coronary Artery

DOG NO.	NO. OF YTTRIUM^{90} PELLETS	PELLET STRENGTH MILLICURIES	TIME (S SACRIFICED) (D DIED)	ECG	POSTMORTEM FINDINGS
643	2	1.0	D 4 days	1. Incomplete data	1. Coronary thrombosis 2. No infarct
831	2	0.5	D 1 mo.	1. Infarction pattern 2. Episodic ventricular tachycardia	1. Thrombosis 2. No gross infarct
665	2	1.0	S 4 mos.	1. Infarction pattern with resolution	1. No thrombosis 2. No gross infarct
757	2	0.5	S 5 mos.	1. In and out of coronary insufficiency pattern	1. Thrombosis 2. No gross infarct
528	2	0.5	S 5 mos.	1. Minimal changes	1. No thrombosis 2. No infarct
227	1	0.8	S 6 mos.	1. No changes	1. No thrombosis 2. No infarct
591	2	1.0	S 6 mos.	1. Infarction pattern with residual 2. Episodic ventricular tachycardia	1. Thrombosis 2. Coronary atherosclerosis 3. Large thin walled infarct

Isolated beads of 1 mc. strength produce a 13 to 14 mm. diameter area of necrosis in the myocardium (and adjacent pericardium and fat) which slowly contracts to approximately 6 to 8 mm. diameter in many months. When the beads are placed about 5 to 8 mm. apart a coalescent infarct is obtained. Figure 1 shows the character of the destroyed myocardium with fibrosis and contraction at 5 months post implantation of a 1.5 mc. radio yttrium pellet.

The production of coronary thrombosis by implantation of the radio yttrium pellets along the anterior descending branch of the left coronary artery has been variable in the present study, but with this background it is probable that a more uniform result can be obtained. The variability of the electrocardiographic and postmortem data should also be less, but changes



Fig 1 Characteristic microscopic findings of a small fibrotic area in the myocardium of dog following implantation of Yttrium^{90} pellets 5 months before



Fig 2 Anterior surface of the heart 6 months after implantation of Yttrium^{90} pellets along the anterior descending branch of the left coronary artery The reaction around the pellets lies close to the base and thinned soft infarcted area lies toward the apex in the diamond between the major branches of this artery

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are sufficient at present to emphasize the importance of producing the coronary obstruction in the intact unanesthetized subject If this experimental subject approaches the clinical counterpart there will continue to be variability of responses and this will be important to control and evaluate adequately Figure 2 shows the anterior surface of the heart of a dog which had had pericoronary implantation of two 1 0 mc Yttrium^{90} pellets 6 months before

SUMMARY

1 Implantation of radioactive yttrium pellets (Y^{90}) into the ventricular myocardium will produce reasonably standard areas of necrosis

2 Implantation of these pellets along the anterior descending branch of the left coronary artery can produce thrombosis the electrocardiographic changes of coronary insufficiency (and infarction) and myocardial infarction

REFERENCES

1 Beck C S A new blood supply to the heart by operation Surg Gyn Obs *61* 407 410 1935

2 Beck C S Revascularization of the heart Ann Surg *128* 854 864 1948

3 Hahn R S and Beck C S Revascularization of the heart A study of mortality and infarcts following multiple coronary artery ligation Circulation N Y *5* 801 809 1952

4 Eckstein R W and Leighninger D S Chronic effects of aorta coronary sinus anastomosis of Beck in dogs Circul Res N Y 2 60 72 1954

5 Rasmussen T Harper I V and Kennedy T The use of a beta ray point source for destruction of the hypophysis in Surgical Forum 1952 Philadelphia W B Saunders 1953 pp 681 686

shielding the magnet from the heart when applying it to the vessels within the chest

The determination of the hematocrit at the end of the procedure is imperative since the sensitivity of the magnet is affected approximately 1 per cent for each 1 per cent change in the hematocrit ratio

Blood flow has been determined and recorded from 12 common carotid arteries 7 femoral arteries 6 external iliac arteries 5 renal arteries 1 axillary artery and 2 brachial arteries

Table 1 Blood Flow Through Intact Human Arteries Range of Values Obtained

ARTERY STUDIED	NUMBER OF DETERMINATIONS	RANGE OF BLOOD FLOW CC/MIN
Common Carotid	12	223 505
Femoral Artery	7	13 618
External Iliac	6	47 221 6
Renal	5	53 803
Axillary	1	209
Brachial	2	30 9 115 8

Table 1 illustrates the range of blood flow values obtained The lowest values for the external iliac and femoral arteries were encountered in patients suffering from occlusive arterial disease One of these patients had a segmental occlusion of the femoral artery and blood flow determinations prior to and after resection of the occluded segment with a vein graft replacement showed an 80 per cent increase in the blood flow at the site of application of the magnet proximally to the occluded segment The lowest value for renal artery blood flow was obtained from a patient who had an atrophic right kidney and a double right renal artery The figures for brachial artery blood flow were obtained from a 10 year old child and a 28 year old male who had no obvious arterial disease

The conditions under which these measurements were done their limited number and the underlying pathological processes involved at the time of operation do not allow us to reach any definite conclusions regarding normal values of blood flow through the arteries studied It will take many more determinations in supposedly normal subjects to establish a set of normal values which at present is not available for this technique It is nevertheless of interest that the figures we have obtained for blood flow through the intact common carotid artery correlate very closely with the values that can be derived from Kety and Schmidt s figures for total cerebral blood flow [3]

The availability of this technique which allows for the determination of blood flow through a surgically exposed vessel without cannulation or trauma offers numerous possibilities to the laboratory and clinical worker It has already been used extensively in animal experimentation [4,5] and we are at present using it to evaluate the results obtained after operations for relief of obliterative arterial disease congenital and acquired lesions of the aorta, and the action of drugs on the peripheral circulation of man

It appears that this procedure places blood flow determinations in the surgical patient on a safe and practical basis thereby adding a new and valuable tool to the experimental and diagnostic armamentarium

REFERENCES

1 Denison A B Jr Spencer M P and Green H D A square wave electromagnetic flowmeter for application to intact blood vessels Circul Res N Y 3 39 1955
2 Spencer M P Denison A B Jr McGuire W I and Myers R J Electromagnetic measurement of blood flow through intact human arteries Am J Med 19 153 1955
3 Kety S S and Schmidt C F The nitrous oxide method for the quantitative determination of cerebral blood flow in man theory procedure and normal values J Clin Invest 27 476 1948
4 Spencer M P Denison A B and Green H D The direct renal vascular effects of epinephrine and norepinephrine before and after adrenergic blockade Circul Res N Y 2 337 1954
5 Spencer M P Denison A B The renal vascular response to vasodepressor sympathomimetics J Pharm Exp Ther (In press)

DYNAMICS OF BLOOD FLOW IN GRAFT DISPROPORTIONS AND IN NORMAL BLOOD VESSELS*

SIGMUND A WESOLOWSKI LESTER R SAUVAGE ROGER S PINC AND CHARLES C FRIES

Experiences in experimental vascular grafting and in extracorporeal heart machines have made us curious about the flow patterns of blood within the major blood vessels in the normal organism and in situations modified by grafting and perfusion. Our preliminary observations have been made using dogs and glass models. This communication summarizes work done in 1955 at the Walter Reed Army Medical Center. It presents our initial observations and relates the germ of an idea to the pathogenesis of atherosclerosis and of atherosclerotic aneurysms.

METHOD

Flow patterns were demonstrated in glass models by the use of visible precipitates and in the living dog by anterograde aortography.

Glass models. Appropriate models were connected at one end by a rubber hose to a water faucet and the other end of the model was connected to a tubing extending upward to a height equivalent to 120 mm Hg hydrostatic pressure. A funnel device at the height of this tubing prevented a siphoning effect. Flows were regulated to 2 to 4 liters per minute. In order to demonstrate flow lines a precipitate of silver chloride was formed within the rubber hose. This was accomplished by dripping concentrated solutions of sodium chloride and silver nitrate through hypodermic needles inserted through the wall of the rubber hose. Adjustment of the flow rates of these 2 solutions resulted in optimal visibility of flow lines. Photographs were taken against a black background using shielded posterior oblique lighting. The models used to demonstrate flow patterns in graft disproportions were of 12 mm I D Pyrex; constrictions were to 6 mm I D while all expansions were to

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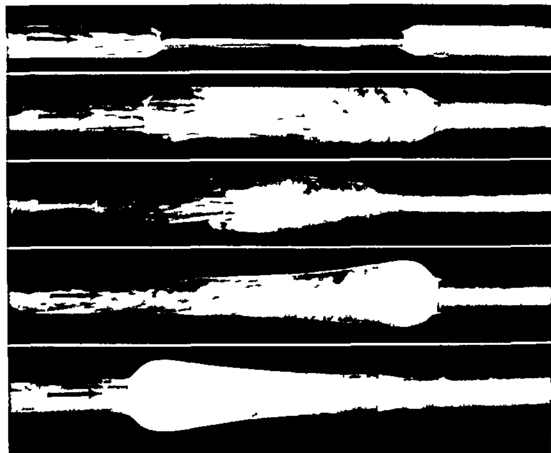


Fig. 1. Demonstration of flow patterns in various graft disproportions using the silver chloride precipitate technique. From top to bottom the glass models are termed: constriction, cylindrical expansion, fusiform expansion, increasing cone expansion and decreasing cone expansion. Note that with every point of expansion turbulent eddy currents form.

21 mm. I.D. The model of the abdominal aorta was of 12 mm. I.D. Pyrex with side branches of 5 mm. I.D. Pyrex.

Dogs. A 1.5 mm. I.D. polyethylene catheter was threaded through the exposed omocervical trunk at the base of the left neck until its tip lay within the descending thoracic aorta. Twenty cc. of 70 per cent Diodrast was injected through this catheter at a rate of from 0.5 to 1.0 cc. per minute. An x-ray of the entire thorax and abdomen using lumbar spine technique was taken near the conclusion of the Diodrast injection.

RESULTS

As can be seen from Figures 1 and 2 the demonstration of flow lines in the glass models was adequate. In all photographs the flow enters from the left. It is a simple matter to distinguish between laminar flow and turbulent flow by direct observation and in photographs. It should be noted that in all situations where the cross sectional diameter of the flow increases there is the presence of turbulence in the segment of increased diameter. This is true whether the increase in diameter is distal to a constriction (post stenotic turbulence) or is an increase in the normal diameter (positive diameter disproportion turbulence). Indeed it can be stated as a hydrodynamic principle that incompressible fluids tend to exhibit turbulent flow when



Fig 2 Demonstration in an abdominal aorta model of turbulence resulting from the side branch effect silver chloride precipitate technique Top (referred to as 2A in text) is a close up of aortic renal artery model showing the back spiralling effect responsible for initiation of turbulence Bottom (referred to as 2B in text) is a model of the abdominal aorta

passing into an expansion It can further be seen from Figure 1 that the shape of the expansion (i e cylindrical fusiform increasing cone or decreasing cone) has little or no effect upon the presence of turbulence although the site of occurrence of the turbulence of nonpulsatile flows varies with the shape of expansion

It was also noted that the rate of flow in a given diameter model had an influence upon the site of turbulence in the expansion In general the greater the flow (the more rapid the velocity) the closer to the origin of the expansion the turbulent flow began Thus one would predict that for a given flow in a given model the introduction of a pulse into the flow would tend to displace the site of onset of turbulent flow toward the origin of the expansion inasmuch as there is an accelerated velocity of flow at the initiation of each pulse Direct observation of pulsatile flows in the glass models demonstrated that indeed the introduction of a pulse displaced the onset of turbulence to the very origin of the expansion

Observations were made in an effort to predict which types of disproportion might favor thrombosis in grafted blood vessels The outstanding variable factor determining thrombosis in grafts and at suture lines is stasis Therefore observations on the velocity of the liquid in contact with the circles of origin of the various types of disproportion (which represent the

Table I. Washing of Anastomotic Lines

MODEL	ANASTOMOTIC LINE	TYPE OF FLOW IN CONTACT	SPEED OF FLOW IN CONTACT
Constriction	Proximal	Compression—Laminar	Rapid and Increasing
	Distal	Laminar and Eddies	Intermediate
Expansion	Proximal	Eddies	Slow
Cylindrical	Distal	Eddies	Rapid
Expansion	Proximal	Laminar and Eddies	Intermediate
Fusiforms	Distal	Eddies	Intermediate and Increasing
Expansion	Proximal	Laminar and Eddies	Intermediate
Increasing Cone	Distal	Eddies	Rapid
Expansion	Proximal	Eddies	Slow
Decreasing Cone	Distal	Eddies	Intermediate

circular suture lines of grafts of like proportions) were made and are summarized in Table I. Survey of Table I will reveal that it is the sudden expansion that favors stasis at the suture line. Within the disproportion there can be stasis and with stasis or decrease in velocity there is an increase in the lateral pressure tending to balloon out the wall of the continuing vessel. The area of maximum stasis is not at the site of obvious onset of turbulence but is proximal to this area where low velocity eddy currents occur that move in a direction opposite to that of the axial flow. These eddies occur at the sites of expansion and are of the severest degree in the abrupt expansion of the normal diameter.

Observations of flows within branched models were made. It was found that in an aortic bifurcation model with the limbs subtending an angle of 15° laminar flow was not interrupted by the presence of the bifurcation. However, when 2 right angle branches were attached 2 cm. apart on opposite sides of a straight tube, a turbulent system was produced. During testing the outputs of both branches and distal end of the straight tube were subject to the same hydrostatic head equivalent to 120 mm. Hg pressure. As can be seen from Figure 2A, turbulence is produced as a result of back spiralling of the flow from the region of the ostium of the distal branch into and through the ostium of the proximal contralateral branch. This spiralling effect is propagated distally as can be seen from Figure 2B. This crude model of the abdominal aorta demonstrates a hydrodynamic turbulence in a location representing that portion of the abdominal aorta between the renal and iliac arteries.

The same effect of side branches producing turbulent flow has been demonstrated in dogs. As can be seen in Figure 3, Diodrast injected anterogradeward into the upper descending thoracic aorta, maintains the slim catheter diameter to the level of the diaphragm. This implies the presence of true axial laminar flow within this portion of the aorta. However, below the diaphragm in the region of origins of the celiac axis, superior mesenteric and renal arteries, the column of dye diffuses throughout the blood within the abdominal aorta resulting in radiographic outlining of the entire width of the aorta. This implies that turbulence and mixing occurs within the



Fig 3 Roentgenogram of an terograde injection of Diodrast into the thoracic aorta of a dog For purposes of reproduction the photograph has been retouched to outline the columns of dye as they appear within the vascular tree The arrow points to the termination of the polyethylene catheter within the descending thoracic aorta Note the axial laminar flow of dye within the thoracic aorta and the spread of dye to the full width of the abdominal aorta

aorta at the sites of the 4 major abdominal branches Studies are now under way to demonstrate this phenomenon in human subjects

DISCUSSION AND CONCLUSIONS

In hydrodynamics any departure from streamline or laminar flow is called turbulent flow The presence of turbulence depends upon the value of the Reynolds number (Re = VD/ν*) When the Reynolds number is large the smooth laminar flow breaks down into a flow in which the paths of the individual particles are erratic with rapid fluctuations of velocity and pressure at any point In the case of flow into an expansion even when the critical value for Reynolds number is not reached peripheral eddy currents can occur in conjunction with an axial laminar flow This is due to loss of kinetic energy of the flow into an expansion which results in inability to overcome the wall friction hence a reverse flow occurs in the region of the wall

It is obvious that hemodynamically the best graft for replacement of a blood vessel defect is one that is isodiametric If one has a choice between a graft which has a smaller diameter and one which has a larger diameter then the smaller diameter graft as recommended by Hughes[3] is probably preferable In such case the expansion with the tendency to peripheral stasis and increased lateral pressure occurs in the distal host vessel which especially in the young individual can withstand these untoward effects more ably than the graft itself Oversized grafts are the worst hemodynam

*Re = Reynolds number V = linear velocity D = diameter of tubing ν = dynamic viscosity

ically for obvious reasons If one has only an oversized venous graft available it is our belief that plication[6] or tailoring is indicated

These studies demonstrate that any expansion in the major blood vessels is unfavorable and therefore aid in understanding the mechanisms of post stenotic aneurysm formation[1] tendency to thrombosis of oversized grafts[5] and the self perpetuating and progressive natures of varices and aneurysms

The brilliant work of Holman[1] whose results were unknown to us at the time of our investigations has clearly demonstrated that aneurysm formation can occur as the result of post stenotic turbulence It is intriguing to consider the possible relationship to vessel wall pathology of the occurrence of turbulent flow occasioned by the presence of side branches in the abdominal aorta It is well accepted that the abdominal aorta is a site of predilection for the occurrence of atherosclerosis and of atherosclerotic aneurysms From the observations presented it is not difficult to explain why atherosclerotic aneurysms should appear more frequently in the abdominal aorta even assuming an aorta homogeneously involved with vessel wall pathology and weakening It is interesting to consider the possible relationship of such hemodynamic effects to the pathogenesis of atherosclerosis itself Such thinking may be especially timely today when most have indicted faulty cholesterol metabolism in the pathogenesis of atherosclerosis this theory leaves much to be desired Pertinent to this discussion is the work of McLetchie[4] who demonstrated typical progression of atheromata in pulmonary artery walls upon intravenous injection of snake venom and thromboplastin to produce multiple small thrombi in rabbits We have observed progression of intravascular mural thrombi through stages of red fibrin fatty degeneration fibrosis and cartilaginous formation which attracts calcium If minute intravascular emboli and thrombi be important in the formation of atheromata then the occurrence of turbulence and eddy current formation distal to the renal vessels as herein demonstrated could account for their localization in this site Such localization of turbulence in the normal aorta also brings to mind the wear and tear theory of atherosclerosis Speculation as to why some people develop atherosclerosis and aneurysms while others do not, raises certain questions what effect do anatomical variations in the sites of origin diameter and direction of the splanchnic and renal arteries have on the occurrence of turbulence in the abdominal aorta? Would unilateral nephrectomy prevent such turbulence? — questions that may be answerable by the judicious use of the simple techniques as outlined

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The authors wish to acknowledge the helpful aid in preparation of the manuscript extended by Dr Shao W Yuan Research Professor of Aeronautical Engineering Polytechnic Institute of Brooklyn

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REFERENCES

1 Holman E On circumscribed dilation of an artery immediately distal to a partially occluding band Post stenotic dilation Surgery *36* 3 24 1954

2 Holman E The development of arterial aneurysms Surg Gyn Obst *100* 599 611 1955

3 Hughes C W Acute vascular trauma in Korean War casualties an analysis of 180 cases Surg Gyn Obst 99 91 100 1954

4 McLetchie N G B The pathogenesis of atheroma Am J Path *28* 413-448 1952

5 Schmitz E J, Kanar E A, Sausage L R, Storer E H, and Harkins H N The influence of diameter di proportion and of length on the incidence of complications in autogenous venous grafts in the abdominal aorta Surgery 33 190 200 1953
6 Schmitz E J Sauvage L R Kanar E A and Harkins H N Plication Arch Surg 66 461 467 1953

PHYSIOLOGIC STUDIES IN EXPERIMENTAL HIGH OUTPUT CARDIAC FAILURE PRODUCED BY AORTIC-CAVAL FISTULA*

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The fact that an arteriovenous aneurysm is characterized by a direct communication between the artery and vein was first described by William Hunter[1] Since his original description there has been an increasing awareness of the fact that the most important changes in this condition occur not at the site of the fistula but in the heart and in the peripheral circulation Cardiac work is increased in proportion to the size of the shunt and in the presence of a large fistula the burden placed upon the circulation may be great In response to this stress the heart is able to compensate for the additional load for a time but later manifestations of failure of the circulation appear

In this study an attempt has been made to follow the cardiodynamics in a group of trained animals before and after the creation of a large aortic-caval fistula From the data obtained it has been possible to establish a pattern of the circulatory adjustments imposed by the shunt and to follow the eventual inadequacy of these compensatory mechanisms with resulting cardiac failure

METHOD

Eighteen adult mongrel dogs were used with each animal serving as its own control Of this group the data from 10 was considered sufficiently complete for comparison The animals were trained to lie quietly while a number of determinations were made in the control state Procaine (1 per cent) was used as a local anesthetic at the site of needle puncture Cardiac output was measured by the dye dilution technique Pressure pulses were recorded by use of a Statham strain gauge and venous pressures were obtained with a saline manometer attached to an indwelling catheter in the superior vena cava Total blood and plasma volumes were determined by the Evans blue dye method From the data obtained calculations were made of (1) stroke volume index in cc /M (stroke volume/surface area M) (2) stroke work index in gm M/M (stroke volume index x (mean arterial pressure – left ventricular end-diastolic pressure) x 13 6) (3) cardiac work index in gm M/M /min (cardiac index x (mean arterial pressure – end-diastolic pressure) x 13 6) and (4) total peripheral resistance (mean arterial pressure x 100 / cardiac output in cc)

Following the repeated determination on different days of normal values for each of the parameters studied a side-to-side aortic caval fistula was

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*Table 1. Survival Time, Causes of Death, and Heart Weight in Dogs Following Aortic Caval Fistula**

NO	SURVIVAL TIME DAYS	FINDINGS AT AUTOPSY	PREDICTED HEART WT GM †	ACTUAL HEART WT GM	HEART WT BODY WT RATIO
1	183	HLE, FLE, ASC, PE	109	180	0.0128
2	21	Death after cardiac biopsy	117	‡	
3	19	Acute PE	135	160	0.0095
4	78	ASC, FLE	115	163	0.0090
5	39	Death after cardiac biopsy	117	‡	
6	162	Ascites, HLE, FLE	138	150	0.0087
7	26	HLE, FLE, ASC, PE	117	141	0.0097
8	5	Death after cardiac biopsy	131	‡	
9	31	Acute PE, HLE	141	224	0.0127
10	Alive				

*Abbreviations: HLE, hind limb edema; FLE, forelimb edema; ASC, ascites; PE, pulmonary edema.

†Calculated from formula of Herrmann.[4]

‡These animals died following cardiac biopsy during the course of studies with radioactive phosphorus. Autopsies were not performed.

created. The anastomosis was approximately 10 mm. in length and was placed between the aorta and inferior vena cava at a point equidistant from the iliac and renal vessels. Previous studies had indicated that these animals tend to develop anemia. Therefore ferrous sulfate was given in daily doses (1.2 gm.). Procaine penicillin was given in maintenance doses of 300,000 units daily and later twice weekly in order to prevent the possible development of bacterial endocarditis.[3] After creation of the fistula the dogs were studied repeatedly for periods up to 183 days (Table 1) and at times as early as the first postoperative day.

Five of the animals were studied in an effort to evaluate the response of the circulatory system under these circumstances to digitalis. For this study control determinations were made in the unanesthetized state immediately before and 45 minutes after the intravenous administration of lanatoside C (0.045 mg./kg.).

RESULTS

General Considerations. The average survival time of those animals which died as a direct result of the effects of the fistula was 83 days. Five animals died in frank cardiac failure with peripheral edema, ascites, and finally pulmonary edema. Three died during punch biopsy procedures on the myocardium. One died with massive ascites and peripheral edema, and 1 continues alive. The preoperative weight of the animals varied between 13.6 and 18.2 kg. (average 16.1 kg.). The average weight increased from 16.1 to 18.3 kg. following operation (animals with ascites were weighed after paracentesis). The hematocrit did not change appreciably and was found to average 43 postoperatively compared with 46 prior to operation.

In these animals the heart definitely enlarged and most of the increase in cardiac size is the result of cardiac dilatation particularly of the right ventricle The values for estimated cardiac weight (calculated by Herrmann's formula heart weight/body weight = 0 00798)[4] are compared with the heart weight at death in Table I It is apparent that there was evidence of cardiac hypertrophy in each instance The average predicted heart weight at death was 131 gm and the actual weight was 170 gm

In general evidence of heart failure appeared by the end of the first month following operation One animal (No 3) represents an example of early onset of heart failure without prior ascites or limb edema Acute pulmonary edema and death occurred 19 days after operation (see Table I) Others illustrate the more usual course with initial onset of limb edema followed by ascites and pulmonary edema

The creation of a large aortic caval fistula results in a marked rise in cardiac output The average control cardiac output was 2 510 This figure rose to 5 610 cc /min after the creation of the fistula The average control heart rate was 110 and after the fistula was created it rose to 150 and remained elevated Terminally it became slower in some animals

The systemic systolic diastolic and mean arterial pressures each fell after the establishment of the fistula The average value for systolic pressure dropped from 158 to 116 mm Hg the diastolic pressure from 102 to 79 mm Hg and the mean pressure from 111 to 96 mm Hg Of particular interest is the effect of the fistula on the left ventricular end diastolic pressure In the early period following the creation of the shunt left ventricular end diastolic pressure was normal or only slightly elevated With time however this value rose and reached figures as high as 32 mm Hg The average control value was 6 9 mm Hg and this rose to 19 7 mm Hg after the fistula The pressure in the superior vena cava rose following the creation of the fistula from an average control value of 2 2 to 7 0 mm Hg but as with left ventricular end diastolic pressure this rise was delayed As has been found in previous investigations[6] the blood volume increased rapidly and tended to reach a plateau The average control value was 1698 cc with a rise to 2212 cc Plasma volume increased from a control level of 767 to 1206 cc

Calculations of cardiac performance demonstrated that the average stroke volume index rose from a control value of 34 to 55 cc /M²/beat The stroke work index also rose from an average of 51 to 59 gm M/M This rise was not as marked as the stroke volume index as a result of two factors (1) the mean arterial pressure was appreciably diminished after the shunt and (2) the left ventricular end diastolic pressure more than doubled its original value Evidence of the marked burden placed upon the circulation is found in the results of calculation of the cardiac work index In every instance the cardiac work rose For the group as a whole the average cardiac work rose from 5250 to 8750 gm M/M /min With the opening of the fistula the peripheral resistance in the systemic arterial circuit fell markedly from a control of 4 9 to 1 7 peripheral resistance units (PRU)

DISCUSSION

A number of investigators have studied the effects of peripheral arteriovenous shunts on the circulation[2-6] Principal among the reference works are the classic studies in the monograph by Holman[6] The data which

constitute the basis of this study have been obtained on trained dogs and in each instance a number of determinations were made prior to the creation of an aortic caval fistula in order that each preparation might serve as its own control

The response of an animal to an arteriovenous shunt is largely dependent upon the size and location of the shunt Previous work has established the fact that an anastomosis between the aorta and vena cava which is greater than 15 mm is very poorly tolerated and constitutes a burden too great for the heart to support [5] The fistulae in this series were kept as nearly possible to 10 mm in length The usual course with such a shunt is the gradual development of evidence of cardiac failure This type of failure is characterized by a *high* cardiac output which may be several times the control value The systolic diastolic and mean arterial pressures were each reduced following the creation of the fistula although there were marked increases in the stroke volume cardiac work and blood and plasma volumes Left ventricular end diastolic pressure was essentially normal in the early postoperative course and rose gradually to values of 30 mm Hg and higher just prior to death This measurement was noted to follow this pattern rather consistently and is believed to represent a reliable index as to the state of cardiac performance

In 5 of the animals a rapid acting digitalis preparation (lanatoside C) was administered in a dosage of 0 015 mg/kg Each of the parameters previously studied was repeated before and after the drug was given intra venously The results indicate that little benefit in cardiac performance was obtained The average changes in cardiac output heart rate mean systemic arterial pressure left ventricular end diastolic pressure central venous pressure stroke work index cardiac work index and peripheral resistance were each within 15 per cent of the values obtained immediately before the administration of lanatoside C Changes of this magnitude were not con sidered significant

SUMMARY

A group of trained dogs has been studied in a controlled unanesthetized state with determinations of a number of cardiodynamic parameters A large arteriovenous fistula (10 mm) was created between the aorta and inferior vena cava and the various studies were repeated at intervals The usual post fistula pattern was characterized by a marked elevation in cardiac out put heart rate blood volume and cardiac work index and a decrease in systemic arterial pressure and total peripheral resistance Left ventricular end diastolic pressure rose slowly but progressively The eventual course was characterized by cardiac failure associated with a high cardiac output edema of the limbs ascites and pulmonary edema Administration of digitalis did not appreciably improve the status of cardiac performance in these animals

REFERENCES

1 Hunter William The history of an aneurysm of the aorta with some remarks on aneurysms in general Med Soc Phys Lond *1* 323 357 1757 Further observations on a particular species of aneurysm Med Soc Phys Lond *2* 390 414 1762

2 Ferguson T B Gregg D E and Shadle O W Effect of blood saline infusion on cardiac performance in normal dogs and dogs with arterio venous fistulas Circul Res N Y *2* 565 1954

3 Lillehei C W Robb J R R and Visscher M B The occurrence of endocarditis with valvular deformities in dogs with arteriovenous fistulae Ann Surg *132* 577 90 1950
4 Herrmann C R Experimental heart disease I Methods of dividing hearts with sectional and proportional weights and ratios for two hundred normal dogs hearts Am Heart J *1* 213 1926
5 Markowitz J Experimental surgery ed 3 Baltimore Williams and Wilkins 1954
6 Holman E E Arterio venous aneurysm New York The MacMillan Co 1937

THE EFFECTS OF THE HUFNAGEL VALVE ON RENAL HEMODYNAMICS IN SUBJECTS WITH AORTIC VALVULAR INSUFFICIENCY*

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While not ideal therapeutically the Hufnagel valve appears to offer clinical improvement in patients with aortic valvular insufficiency The reduction in heart size and increase in exercise tolerance generally observed following insertion of the plastic valve in the aorta just distal to the left subclavian artery cannot be duplicated by medical treatment [1] Some of the dramatic alterations in the hemodynamics proximal to the valve have been studied previously Thus Rose reported an increase in cardiac output in 6 of 8 patients and reduction in mean circulation time in 7 of 8 patients McKusick constructed models in which the effect of the valve produced a reduction in total volume of regurgitation [3] Both Rose and McKusick demonstrated reduction in diastolic pressure recorded from the segment proximal to the Hufnagel valve in patients with aortic insufficiency Still more data is needed on the hemodynamics of the regurgitant arterial segment proximal to the prosthetic valve Of great interest would be measurements of cerebral and coronary blood flow

This study however is based on observations of hemodynamics involving the nonregurgitant arterial segment distal to the Hufnagel valve The effects of the Hufnagel valve on renal hemodynamics were recorded in 8 patients with aortic valvular insufficiency The investigation was prompted by the improvement in a patient exhibiting chronic uremia prior to insertion of a Hufnagel valve Improvement in renal function by more nearly normal renal arterial dynamics would certainly add supplemental indication for use of the Hufnagel valve

METHOD

Observations on renal function and on water and electrolyte excretion were made on 8 patients with aortic valvular insufficiency Follow up studies were conducted following insertion of the Hufnagel valve Renal function determinations were made within 48 hours prior to operation at a time

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when cardiac compensation was as optimum as could be effected by strict medical management. The subsequent data were derived from studies carried out during the seventh to fourteenth day after operation. Renal function was determined using inulin clearance as a measure of glomerular filtration rate (GFR) and low concentrations (2 to 4 mg per cent) of para aminohippurate to measure renal plasma flow (RPF) employing methods and techniques previously described.[4] Venous blood collected from an antecubital vein was used for all analyses. Figures listed in the table for these studies are averages of three 10 minute collection periods. Auscultatory blood pressures in the arm and leg listed in Table 2 are averages of 3 readings taken during the study. Preoperative pulse rates listed in Table 1 are averages of multiple recordings taken during the resting state over a 2 to 5 day period. The postoperative pulse rates were obtained from averages of multiple observations in the resting state after the seventh post operative day.

RESULTS

Seven of the 8 patients showed moderate to severe depression in renal function before operation. The 8 individuals were again studied during the seventh to fourteenth day after operation. In 5 patients multiple follow up studies were performed ranging from the first to the sixtieth day after operation. During the first and second days there was no improvement or even a slight depression in renal function. Thereafter a progressive improvement in glomerular filtration and renal plasma flow occurred which reached maximum levels by the fourteenth day after insertion of the valve.

The average age for the group was 44 years. There was but 1 female. The etiology of the aortic valvular insufficiency was due to syphilis in 5 patients and rheumatic fever in 3 patients. Prior to operation 1 patient was in uremia with a blood urea nitrogen of 100 mg per cent. In another patient the blood urea nitrogen was elevated to 42 mg per cent before

Table 1 Clinical Summary of Patients with Aortic Insufficiency Treated with Insertion of the Hufnagel Valve

PATIENT	AGE	SEX	ETIOLOGY	PREOPERATIVE BUN	PREOPERATIVE URINALYSIS			HEMATOCRIT		PULSE	
					SPG	PROTEIN mg %	MICRO	C	S	C	S
1 R M	51	M	Syphilis	100	1 010	10	Occ hyalin	41	30	78	96
2 R D	31	M	R H D		1 024	100	Occ hyalin	41	38	72	74
3 S W	54	M	Syphilis	42	1 024	10	Few hyalin	47	48	81	86
4 H C	46	M	Syphilis	18	1 020	5	Occ hyalin	49	44	56	50
5 J C	48	M	Syphilis	24	1 020	400	Few W B C	43	44	81	65
6 E B	38	F	R H D	15	1 016	0	Few W B C	45	44	80	77
7 S H	46	M	R H D		1 016	0	Few W B C	39	37	87	85
8 H W	40	M	Syphilis	22	1 020	0	Few W B C	40	40	77	75
Mean —	44							43	41	77	76

Foot Notes C — Preoperative control
S — 7 to 14 days after insertion of the Hufnagel valve
R H D — Rheumatic Heart Disease

Table 2 The Effects of the Hufnagel Valve on Blood Pressure, Renal Plasma Flow and Renal Blood Flow in Patients with Aortic Insufficiency

PATIENT	AUSCULTATORY BLOOD PRESSURE ARM mm/Hg		LEG mm/Hg	RENAL PLASMA FLOW ml/min		RENAL BLOOD FLOW ml/min	
	C	S	S	C	S	C	S
1 R M	190/0	300/0	260/135	187	210	317	343
2 R D	260/0	300/0	160/40	715	661	1263	1071
3 S W	160/80	160/0	180/110	142	159	268	306
4 H C	170/70	190/0	215/90	342	425	671	759
J C	200/0	180/0	160/80	652	516	1144	921
6 F B	146/40	200/0	180/90	457	403	795	720
7 S H	220/0	180/0	230/140	439	435	720	690
8 H W	150/40	170/0	160/100	457	362	702	603
MEAN	185/29	210/0	193/102	425	401	745	677
Per cent of Control		114/			94		91

c — Preoperative control (average of 3 ten minute periods)
s — 7 to 14 days after insertion of the Hufnagel valve

$$\text{Renal Blood Flow} = \frac{\text{Renal Plasma Flow}}{1 - \text{Hematocrit}}$$

Table 3 The Effects of the Hufnagel Valve on Glomerular Filtration Rate Water and Electrolyte Excretion in Patients with Aortic Insufficiency

PATIENT	GLOMERULAR FILTRATION RATE ml/min		URINE VOLUME ml/min		PLASMA SODIUM mEq/Liter		PLASMA POTASSIUM mEq/Liter		SODIUM EXCRETION mEq/min		POTASSIUM EXCRETION mEq/min	
	C	S	C	S	C	S	C	S	C	S	C	S
1 R M	16	36	9	6 0	131	140	5 7	4 5	05	03	04	04
2 R D	125	98	4 6	4	141	138	4 9	4 4	06	01	08	05
3 S W	33	39	10 8	8	125	141	5 1	4 1	03	04	01	06
4 H C	82	96	1 5	1 9	137	140	3 6	3 2	07	08	05	04
5 J C	67	102	5 9	3 4	146	141	5 4	3 7	29	21	04	04
6 F B	72	77	4 5	3 0	135	144	4 0	3 9	13	14	06	06
7 S H	75	100	4 7	9 6	144	139	5.2	4 6	07	14	08	07
8 H W	62	86	4 4	8 8	154	120	4 3	3 8	17	10	07	05
MEAN	67	79	4 7	4.2	140	138	4 8	4 0	11	09	05	05
Per cent of Control		118		89		99		83		82		100
P Value*		<0 1		N S		N S		<0 01		N.S		N.S
Without R D		<0 01										

c — Preoperative control
s — 7 to 14 days after insertion of the Hufnagel valve
NS — P < 0.50
* — $t = x/\sqrt{n\ (n\ 1)}$
s 2

operation. Albumin was present in the urine of 5 cases and hyalin casts were seen in 4 patients. There was no significant change in hematocrit or pulse rate following insertion of the Hufnagel valve. Auscultatory brachial arterial pressure showed the characteristic fall to zero postoperatively in the 5 patients with audible preoperative diastolic pressures. Five subjects had an increased systolic pressure at the time of the follow-up renal clearance study. Except for 1 patient (R.D.) all revealed moderate to severe depression in preoperative glomerular filtration rate. All of these patients showed an increase in glomerular filtration rate following insertion of the Hufnagel valve. This increase in glomerular filtration rate was statistically significant ($p<0.01$). R.D. with a normal preoperative glomerular filtration rate was somewhat reduced by the procedure. For the whole group there was a mean increase of 18 per cent ($p<0.1$). There were no significant changes in renal plasma flow, renal blood flow or urine volume. Plasma sodium and excretion of sodium and potassium remained unchanged. Mean plasma potassium, however, was slightly reduced postoperatively (17 per cent $p<0.01$).

DISCUSSION

These observations indicate that renal function is characteristically depressed in the presence of clinically overt aortic valvular insufficiency. Glomerular filtration rate in 7 patients was below the normal range. In 4 of these the filtration rate was quite abnormally low (16 to 67 ml./min.). Only 1 subject (R.D.) had a normal preoperative glomerular filtration rate and conversely was the only case with a postoperative reduction in this function. The remaining 6 patients showed minimal to striking improvement ($p<0.01$). Subnormal renal blood flow values were present in 6 patients preoperatively. Two patients (R.D. and F.B.) had normal renal blood flows. There was no significant change in renal blood flow after insertion of the Hufnagel valve. The postoperative mean per cent of control was 91 per cent ($p<0.5$). It would seem logical to conclude there

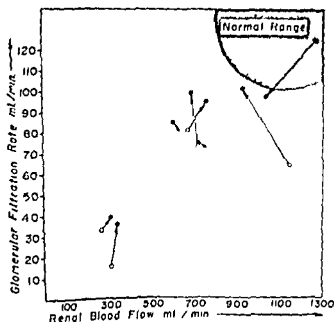


Fig. 1. Comparison of preoperative (o) and postoperative (•) observations on renal function. The arrow (→) connects these observations on each patient and indicates the direction of change after insertion of the Hufnagel valve.

fore that the improvement in glomerular filtration rate results from the elevation of the mean diastolic blood pressure distal to the valve rather than from the direct effects of increased cardiac output

The cause for the 17 per cent postoperative reduction in plasma potassium ($p<0.01$) is obscure Though variable red cell destruction by the newly inserted valve would tend to elevate extracellular potassium The patients had all been on diets beginning the first postoperative day and in general these diets were less salt restrictive than the preoperative diets It would be perhaps hopeful to assume that the lowering in plasma potassium was due to improved renal function

SUMMARY AND CONCLUSIONS

1 Renal function studies have been made in patients suffering with aortic valvular insufficiency Repeat observations were made following insertion of the Hufnagel valve

2 Seven of 8 patients showed moderate to severe depression in renal function prior to operation

3 All patients with low glomerular filtration rates responded to insertion of the Hufnagel valve with improved filtration rates

4 Insertion of the Hufnagel valve did not significantly alter renal blood flow Water and electrolyte excretion were not changed A small but significant reduction in plasma potassium was observed The plasma sodium remained unchanged

5 The demonstration of depressed renal function in aortic valvular insufficiency and the improvement in glomerular filtration rate following insertion of the Hufnagel valve adds new impetus to the operation

REFERENCES

1 Hufnagel C A Harvey W P Rabil P J and McDermott T F Surgical correction of aortic insufficiency Surgery 35 676 1954

2 Rose J C Hufnagel C A Freis E D Harvey W P and Partenope E A The hemodynamic alterations produced by a plastic vascular prothesis for severe aortic insufficiency in man J Clin Invest 33 891 1954

3 McKusick V A Hahn D P Brayshaw J R and Humphries J O Some hemodynamic effects of the Hufnagel operation for aortic regurgitation Bull Johns Hopkins Hosp 95 322 337 1954

4 Moyer J H and Mills L C Hexamethonium—its effect on glomerular filtration rate maximal tubular function and renal excretion of electrolytes J Clin Invest 32 172 1953

5 Smith H W The kidney Structure and function in health and disease New York Oxford University Press 1951 pp 544 545

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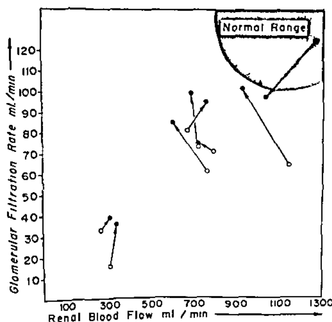


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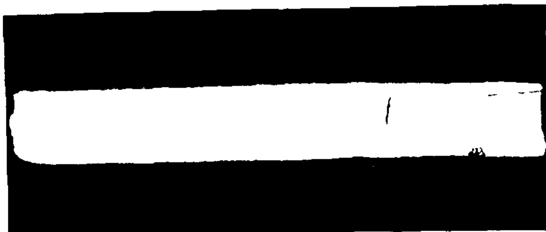


Fig. 1. Collagen tube produced by enzymatic digestion of bovine carotid artery with 1 per cent ficin. Wall partly cut away to show smooth inner surface.

ment of the abdominal aorta removed below the renal arteries. Either a collagen tube or untreated heterograft was used to bridge the defect and sutured into place with 5-0 arterial silk applied as inner everting and outer over-and-over layers. An attempt was made to match donor and host vascular diameters. The implants varied in length from 2.5 to 5.2 cm., averaging 4.0 cm. All animals received penicillin postoperatively.

Aortograms using 35 per cent sodium urokon (sodium acetrizoate) have been carried out by direct aortic puncture in all dogs in an effort to preserve most of the animals for long-term observations. Selected animals have been studied after sacrifice along with those succumbing spontaneously during investigation. The period of observation has varied from 3 months to 1 year, most of the animals having been observed for more than 6 months.

Tissue reactions to control and ficin-digested material were investigated in 12 Wistar rats weighing 250 to 300 gm.[3] Using aseptic technique, a small transverse incision was made in the interscapular region of each animal. Subcutaneous tunnels were fashioned by introducing a large hemostat in the direction of both flanks. Arterial strips measuring approximately 3 cm. in length and 1.5 mm. in width were implanted by grasping one end with a hemostat and depositing it into the distal end of the preformed tunnel away from the skin incision. The incision was closed with Michel clips. The rats were sacrificed at 11, 25 and 40 days postoperatively and tissue sections stained with hematoxylin-eosin and with Verhoeff's elastic tissue stain.

RESULTS

The results reported on 11 dogs subjected to aortic grafting procedures are early results, the animals having been observed for periods varying from 3 months to 1 year. The observations, mostly based on aortography, are summarized in Tables 1 and 2.

It is of interest that thrombosis resulted in more than half of those animals in which a "poor fit" was noted in the protocol at the time of grafting and that 3 of the 4 thrombosed grafts were in the "poor fit" category. A control series of 8 animals, too small for serious analysis, was employed in this phase of the investigation mainly to provide material for histologic comparison.

THE USE OF SEGMENTAL ARTERIAL IMPLANTS PREPARED BY ENZYMATIC MODIFICATION OF HETEROLOGOUS BLOOD VESSELS*

NORMAN ROSENBERG, E. R. L. GAUGHRAN, JOHN HENDERSON, GEOFFREY H. LORD AND JOCELYN FIELDING DOUGLAS

The problems of obtaining an adequate and varied supply of arterial homografts and the knowledge that degenerative changes eventually appear in these grafts impose limitations on their use as arterial substitutes. With plastic materials the technical problems of tailoring, seamless weaving, constructing the Y tube, and wrinkle thrombosis are evident.

Arterial heterografts, on the other hand, are readily available in suitable sizes and configurations but have in most laboratories given rise to a higher incidence of failures than have homografts. The poorer results have been attributed to an accelerated immunologic response on the part of the host leading to thrombosis, rupture or the more frequent occurrence of aneurysm in the experimental animal. It is important to note, however, a definite incidence of reported success with heterograft material.[1,2]

In this investigation, arterial heterografts were stripped of most of their contained parenchymatous proteins by controlled enzymatic digestion, leaving a tubular prosthesis composed mostly of collagen. These tubes were employed as aortic substitutes in the hope that with most of their immunologically reactive proteins removed, the implants would be better *tolerated by the host while yet maintaining their effectiveness as arterial* substitutes.

METHOD

Bovine carotid arteries, freshly removed from animals at slaughter, were trimmed of adherent tissue and thoroughly washed. The vessels were then subjected to digestion in a solution of 1 per cent ficin (Merck) for 3 hours at a temperature of 37 C, using standard phosphate citrate buffer to keep the pH at 5.0 or above. They were then washed in running water for one half hour, cut into usable sections and further trimmed by hand to remove any superfluous matter. The resulting collagenous tubes were placed on glass rods and hardened somewhat by immersion in 1 per cent formalin for 18 hours. The product was again washed for one half hour and tested for leaks at 240 mm. Hg pressure. All tributaries were carefully tied with 3-0 silk at that time. The diameters of the vessels were recorded at 120 mm. Hg and each was placed separately in 50 per cent ethanol containing 1 per cent propylene oxide. A ratio of 1 gm. of artery to at least 30 ml. of sterilizing fluid was maintained, resulting in a sterile material after 5 days. The vessels were either stored in this solution or aseptically removed for lyophilization and storage in a dry state. Fig. 1 shows the appearance of these ficin treated vessels. Untreated bovine carotid arteries similarly sterilized and stored constituted control material.

Forty-one large dogs of various breeds (averaging 23 kg. in weight) were subjected to laparotomy under intravenous nembutal anesthesia and a seg

*From The Vascular Services of Middlesex and St. Peters General Hospitals and The Johnson and Johnson Research Foundation, New Brunswick, N. J.

graft showed longitudinal bands of elastic tissue undergoing fragmentation and lined by an incomplete layer of degenerating muscle Reactive fibroplasia provided support on the outer surface while the inner aspect was covered by a flat mural thrombus The cellular reaction to the prosthesis was intense This was especially so in areas adjacent to degenerating muscle and elastica At 151 days, fragmentation of the elastic fibers was more extensive Degenerative and reparative changes were recognized in the media A new intima consisting of a thin layer of fibrous tissue originated at the suture lines but extended out over the graft for a much shorter distance than in comparable ficin-digested implants A flat mural thrombus covered the central portion of the graft Calcific deposits were observed in the degenerating media and a relatively large plaque of metaplastic cartilage was present in one area on the intimal surface The adventitial layer was heavily infiltrated with mononuclear phagocytes and moderate numbers of giant cells

Even after these limited observations it became apparent that the collagen tube gave rise to far less intense and less prolonged tissue reaction and presented fewer degenerative changes

Subcutaneous implantation of control and treated arterial strips in the rat gave comparable results At 11 days the inflammatory reaction to the control strip approached that of suppuration while the treated implant showed heavy infiltration by fibroblasts with a minimal inflammatory reaction By 25 days the control had lost its residual muscle and a moderate chronic inflammatory reaction was noted This reaction was still to be seen at 40 days at which time the implant was still identifiable In the treated implant at 25 days the arterial strip was organized to the point where it was difficult to distinguish it from the surrounding host tissues

DISCUSSION

Ficin treated arterial heterografts are strong tubes composed mostly of collagen white in color, with a smooth inner lining and somewhat roughened exterior Few if any elastic fibers are present The collagen is present in a tightly woven mesh which does not leak blood or even air under pressure through its microscopic interstices The handling qualities are fair and have prospects of even further improvement with processing modifications now under study It seems to incite far less inflammatory reaction both in the dog when used as an arterial substitute and in the rat when implanted subcutaneously, than does untreated heterograft material These histologic observations would seem to incriminate the parenchymatous and easily digested proteins of the parent heterograft as responsible for a considerable portion of the noxious tissue reaction elicited

Long term observations are required and are being carried out to determine whether these implants will dilate and whether they will eventually show degenerative changes comparable to those found in untreated heterografts and even in some homografts

SUMMARY

Heterologous arteries of bovine origin have been subjected to enzymatic digestion to produce collagen tubes These have been implanted into the abdominal aorta of dogs as segmental arterial replacements with some success

Table 1

	NO OF DOGS	SUCCESSFUL *		NO OF FAILURES		
		NO	%	TOTAL	<2 WKS	>2 WKS
Ficin digested implants	33	23	70%	10	6	4

*i e patent and not dilated at autopsy or recent aortography

Table 2

FAILURES — 10

I—Failed for Reasons Definitely Technical (2 Dogs) and Probably Technical (5 Dogs) — 7†

TECHNICAL DEFECTS —	RESULTS —
3 poor fitting prostheses	Thrombosis noted on days 1 118 and 115
3 defective preparations	
1 calf implant with marked focal thinning	Aneurysm noted on day 35
1 incompletely digested	Wall necrosis and rupture on day 9
1 1 hour formalin mushy	Rupture on day 3
1 old hematoma and infection about graft	Rupture on day 5
II—Unexpected failures — 3	
	1 Thrombosis noted on day 145
	2 Rupture at suture line on days 10 and 14

†Reasons given for failure in this group were those gleaned from a review of protocols or photographs taken at operation

OBSERVATIONS

Collagen tubes of bovine arterial origin when used to replace excised aortic segments in the dog became quickly coated on their inner aspects with a thin flat finely granular mural thrombus Cellular reaction to the implant was not severe and at 14 days was mainly at the suture lines At 21 days the wedge thrombi at the anastomoses were completely organized with infiltration of the thrombi by fibroblasts The central portion of the graft was still covered by a thin flat fibrin layer Cellular reaction was moderate at the suture lines and minimal throughout the remainder of the graft At 30 days the graft was supported by a much thicker layer of new fibrous tissue Cellular reaction was minimal At 51 days recognition of the original prosthesis was possible only at the center of the graft in other areas being completely replaced by new fibrous tissue Pseudoendothelium composed of differentiated fibroblasts extended 6 to 8 mm towards the middle of the graft At 90 days the original prosthesis could no longer be identified The entire graft consisted of new collagenous tissue and its wall was much thicker than that of the host aorta The thin flat mural thrombus over the central portion of the graft was in process of infiltration by fibroblasts At 119 days the entire prosthesis was fibrosed except for a small *organizing central mural thrombus*

Two controls each an untreated bovine arterial heterograft were examined after implantation into the canine aorta At 70 days one such

Group III

(a) Left spleno renal arterial anastomosis
(b) Resection of aortic segment including origin of both renal arteries with reimplantation (reversed)
(c) Right renal arterial anastomosis into reimplanted segment of aorta

A left paramedian muscle splitting abdominal *incision* was *used in* all dogs The splenic and renal arteries were mobilized and prepared and the proximal end of the splenic artery was anastomosed to the distal end of the left renal artery with continuous 5 0 silk sutures interrupted at the lateral angles The technique of the anastomoses has been described in detail in a previous publication [1]

In Group I the abdominal aorta was exposed and mobilized for a distance of 3 cm below the renal arteries and 1½ cm above The right renal artery was mobilized ligated at its origin clamped distally with a bull dog clamp sectioned and implanted into an opening made in the abdominal aorta above the anticipated proximal line of section The aorta was occluded with 2 Potts clamps and the end to side renal aortic anastomosis was *done with a* single continuous 5 0 silk atraumatic suture running from the posterior wall anteriorly The clamps were then replaced below the right renal aortic anastomosis and the segment of aorta containing the renal artery origins was resected this was reversed and reimplanted using the same suture technique as in the spleno left renal arterial anastomosis

In Group II both kidneys were revascularized by the same technique In this group the aorta was not resected

In Group III following division of the right renal artery the segment of aorta containing the renal artery origins was resected reversed and the proximal aorto aortic anastomosis performed The right renal artery was *anastomosed into the reversed aortic segment and then the distal aorto aortic* anastomosis was completed The procedures are summarized in Figure 1

Special Considerations (a) It was noted that a slit in the aorta for the right renal aortic anastomosis was not adequate because of the tendency of the edges to fall together and occlude the lumen This was overcome by excising an *ellipse* of tissue from the aorta or using the base of a pre existing vessel in the graft (b) In Group III in a preliminary attempt to shorten the period of right renal ischemia the proximal aortic clamp was moved below the right renal and proximal aortic anastomoses after the right renal circulation had been re established the distal aortic anastomosis was com pleted This procedure was discontinued because of excessive hemorrhage from the proximal anastomoses while the distal *one* was *being completed*

RESULTS

Twenty seven dogs were operated upon with 14 survivors The detailed data are presented in Tables 1 and 2 The high mortality in Group I can be attributed in part to the problems involved in the development of *technique In the surviving* dogs with patent anastomoses the average time for performance of the left renal anastomosis was 24 minutes the right renal anastomosis was 26 minutes and the total time for the 2 aorto aortic anasto moses was 36 minutes One hundred five minutes was the maximum time of arterial occlusion for a single kidney and the abdominal aorta This dog survived with grossly normal kidneys and no residua due to aortic occlusion

Tissue reactions to enzyme treated arteries are significantly less intense than those seen after implantation of untreated controls

REFERENCES

1 Hufnagel C A et al A method for the preservation of arterial homo and hetero grafts in Surgical Forum 1952 Philadelphia W B Saunders Co 1953 pp 162 168
2 Hufnagel C A In Henry Ford Hospital International Symposium on Cardiovascular Surgery Philadelphia W B Saunders Co 1955 p 528
3 Lattes R and Frantz V K Absorbable sponge tests Ann Surg 121 894 896 1945

AVOIDANCE OF RENAL ISCHEMIA DURING RESECTION OF THE ABDOMINAL AORTA INCLUDING THE RENAL ARTERIES*

ELLIOTT S HURWITT STANLEY L ALTMAN BERNARD SEIDENBERG
AND HENRY HAIMOVICI

The hazard of renal ischemia has retarded application of the resectional treatment of aneurysms to those involving the renal artery bearing portion of the aorta Experiments previously reported from this laboratory[1] have demonstrated that dogs with a left splenorenal arterial anastomosis maintain adequate renal function despite a right nephrectomy In the present study removal and replacement of the aorta including the origins of the renal arteries have been accomplished in a series of dogs with no period of simultaneous bilateral renal arterial occlusion This was accomplished by a preliminary left splenorenal arterial shunt while the right kidney was intact Reimplantation of the right renal artery into either the aorta or the graft was then effected in the presence of a functioning left kidney

METHOD

Mongrel dogs were used weighing from 30 to 60 lbs All operations were performed under general anesthesia produced by a veterinarian solution of nembutal administered intravenously The experimental procedure was divided into 3 major groups

Group I

(a) Left splenorenal arterial anastomosis
(b) Right renal arterial anastomosis into the aorta above the aortic segment to be resected
(c) Resection of aortic segment including origins of both renal arteries with reimplantation (reversed)

Group II

(a) Left splenorenal arterial anastomosis
(b) Right renal arterial anastomosis into aorta
(c) No aortic resection performed

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*From the Surgical Research Laboratory and the Surgical Division the Montefiore Hospital New York City New York The authors gratefully acknowledge the cooperation of Mr Antol Herskovitz Medical Photographer and the technical assistance of Mr Ruthven Ferreira and Mr Alphonso Ivan Henry

Table 1 Left Splenorenal and Right Renal Aortic Arterial Anastomoses

DOG	DURATION OF ARTERIAL OCCLUSION (MINUTES) LEFT RENAL	RIGHT RENAL	AORTA	SURVIVAL (DAYS)	CAUSE OF DEATH	STATUS OF ANASTOMOSES LEFT RENAL	RIGHT RENAL	AORTA	COMMENT
					GROUP I — AORTA RESECTED				
A1	1[illegible]	30	57	188	Sacrificed	Patent	Occluded	Patent	Right kidney atrophic
A2	15	26	53	1	Hepatitis	Partially Occluded	Occluded	Occluded	
A3	20	29	10	4	Pneumonia	Patent	Patent	Patent	
A4	18	41	50	2	Uremia	Occluded	Occluded	Occluded	
A5	18	25	41	1	Pneumonia	Occluded	Patent	Patent	Gastric Dilatation
A6	11	37	10	11	Uremia	Occluded	Occluded	Patent	Peritonitis Right kidney atrophic
A7	14	20	10	149	Sacrificed	Occluded	Patent	Patent	Left kidney atrophic
A8	36	50	37	117	Sacrificed	Patent	Occluded	Patent	Right kidney atrophic
A9	14	26	36	5	Uremia	Patent	Occluded	Patent	Right kidney atrophic
A10	18	28	44	4	Uremia	Occluded	Occluded	Occluded	
A11	12	15	30	5	Uremia	Occluded	Occluded	Patent	
A12	18	32	30	1	Hemorrhage	Patent	Patent	Patent	
A13	10	25	43	120	Sacrificed	Patent	Patent	Patent	
					GROUP II — AORTA NOT RESECTED				
A14	57	25	45	114	Sacrificed	Patent	Patent	Patent	
A15	14	28	21	118	Sacrificed		Patent	Patent	Left nephrectomy—renal vein injury
A16	39	41	10	1	Uremia	Occluded	Occluded	Patent	
A17	15	24	21	105	Sacrificed	Patent	Patent	Patent	
A18	39	15	26	99	Sacrificed	Patent	Patent	Patent	
A19	16	36	33	97	Sacrificed	Patent	Patent	Patent	
A20	16	23	25	1	Pneumonia	Patent	Patent	Patent	

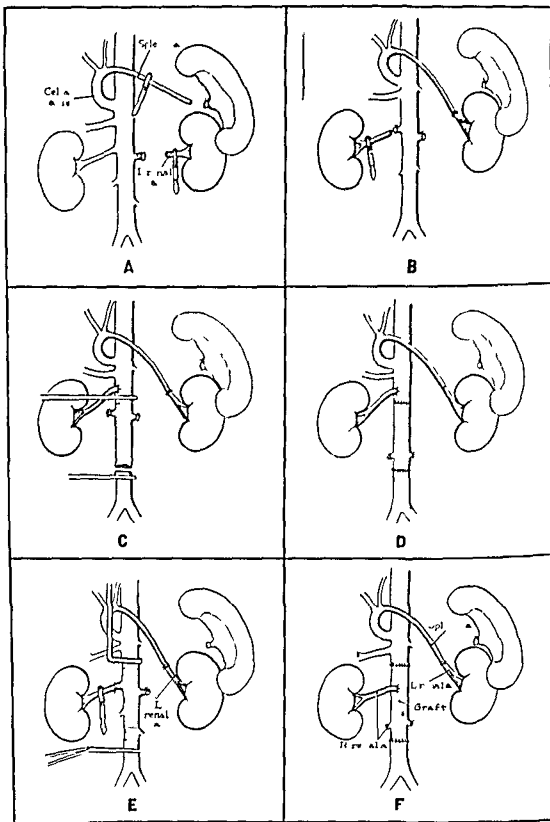


Fig 1 Avoidance of renal ischemia during aortic resection by left splenorenal arterial anastomosis (A B) followed by implantation of right renal artery either into aorta (C D) or into graft (F I)

DISCUSSION

Resection of the renal arteries with the aorta should extend the scope of surgery for lesions hitherto considered inoperable. The current investigation provides a means for carrying this out in such a way that one kidney is functioning at all times. Other approaches to the problem involve the insertion of homografts including the renal arteries and the possible application of hypotension and hypothermia[2] both of these methods entail a period of total bilateral renal vascular occlusion. Total occlusion of the thoracic aorta for 68 minutes was reported in one case[2] without visceral damage and Movius has described occlusion of the right renal artery for 80 minutes.[3]

Application of the present method to human cases would appear justified on the basis of these experimental results. The operator must be on the lookout for multiple renal arteries which are not uncommon either in dogs or in patients.[4] While success might be obtained with only one functioning kidney this should not be the goal. Anastomosis of the right renal artery to an elliptical defect or a branch of the graft and protecting the shunt from the full impact of aortic flow by releasing the aortic clamps after all of the suture lines had been completed were important technical maneuvers. The various steps in this method may all be performed at one operation or in stages with the left splenorenal arterial shunt as the first stage. On April 19 1955 a left splenorenal arterial anastomosis was constructed in a 41 year old man with ascending thrombosis of the aorta and a previously unsuccessful resection and homograft replacement. The thrombosis extended from the femoral arteries to just below the origin of the renal arteries and the threat of renal vascular occlusion seemed imminent. Intravenous pyelograms 2 and 3 months postoperatively revealed both kidneys to be functioning well with sharper visualization of the left kidney on both occasions.

Intravenous pyelogram 5 months postoperatively showed non function of the right kidney suggesting occlusion of the right renal artery and survival dependent on the left splenorenal arterial anastomosis.

The splenic artery has been employed previously to bypass a low aortic coarctation[5] and aortic aneurysms.[6] It is believed that the present case represents the first use of the splenic artery to replace a visceral artery. Splenorenal arterial anastomosis provides a preferable means for dealing with hypertension due to renal artery occlusion than thromboendarterectomy[7] or nephrectomy[8,9] and may be applied when the origins of the renal arteries are narrowed by a congenital coarctation as described by Fisher and Corcoran.[10] The availability of the splenic artery for replacement of visceral arteries other than the renal has already been indicated.[1]

SUMMARY

1. A technique has been described for resection of the renal artery bearing portion of the aorta with no period of simultaneous bilateral renal arterial occlusion.

2. A left splenorenal arterial anastomosis may be followed by implantation of the right renal artery into the aorta or the graft.

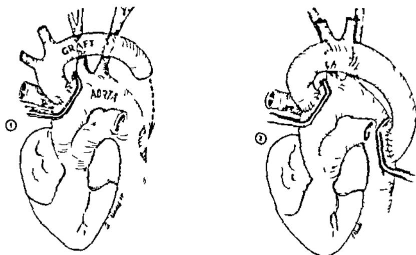
3. A successful left splenorenal arterial shunt was performed for ascending thrombosis of the aorta.

Table 2 Left Splenorenal and Right Renal Aortic Graft Arterial Anastomoses

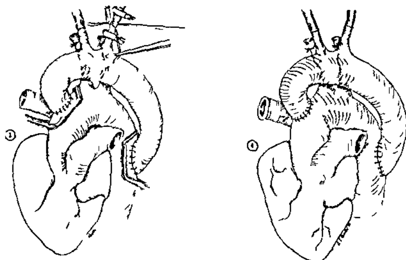
DOG	DURATION OF ARTERIAL OCCLUSION (MINUTES) LEFT RENAL	RIGHT RENAL	AORTA	SURVIVAL (DAYS)	CAUSE OF DEATH	STATUS OF ANASTOMOSES LEFT RENAL	RIGHT RENAL	AORTA	COMMENT
					GROUP III				
A25	15	55	55	59	Sacrificed	Patent	Occluded	Patent	Right kidney atrophic
A26	18	105	105	58	Sacrificed	Patent	Patent	Patent	
A27	15	60	60	54	Sacrificed	Patent	Patent	Patent	
A28	12	58	58	61	Sacrificed	Patent	Patent	Patent	
A29	24	61	61	10	Empyema	Occluded	Patent	Patent	
A30	15	60	60	7	Uremia	Occluded	Occluded	Patent	
A31	24	41	41	36	Sacrificed	Patent	Patent	Patent	

The anastomosis was done with continuous 5 0 arterial silk following the placement of the proximal and distal stay sutures After testing the proximal and distal suture line a similar anastomosis was performed at the level of the diaphragm Upon completion of the latter blood was allowed to flow through the shunt The intervening aorta was then resected between Potts ductus clamps and the divided ends oversewn with arterial silk* Retraction of the aorta allowed the tented graft to straighten out The chest was closed Penicillin was given for 10 days Autopsy was performed at the time of death or sacrifice

Resection of the Aortic Arch A method was devised for the resection of the arch in which a permanent shunt was used In the supine position the thorax was opened through a transsternal incision in the fourth interspace The pericardium was widely incised from the base of the heart to its uppermost reflection at the origin of the 2 brachiocephalic arteries After preliminary isolation of the left phrenic and vagus nerves the entire arch of the aorta and its branches were mobilized The ascending aorta was partially occluded with a modified Beck clamp and the previously prepared graft was



Figs 1 & 2 Aortic arch completely mobilized The graft is anastomosed to the ascending aorta end to side with continuous 5 0 silk



Figs 3 & 4 Complete replacement of the arch with the graft The shaded area is resected and the divided aorta oversewn with 5 0 arterial silk

REFERENCES

1. Hurwitt E S, Altman S F, Borow M and Rosenblatt M Intra abdominal arterial anastomoses Surgery 37 1015 1955
2. Julian O C, Grove W J, Dye W S, Sadove M S, Javid H and Rose R F Hypotension and hypothermia in surgery of thoracic aorta Arch Surg 70 629 1955
3. Movius H J II Resection of abdominal arteriosclerotic aneurysm Am J Surg 90 298 1955
4. Anson B J and Kurth L E Common variations in the renal blood supply Surg Gyn Obst 100 156 1955
5. Clenn F, Keefer E B C, Speer D S and Dotter C T Coarctation of the lower thoracic and abdominal aorta immediately proximal to celiac axis Surg Gyn Obst 94 561 1952
6. Freeman N E and Leeds F H Resection of aneurysms of the abdominal aorta with anastomosis of the splenic to the left iliac artery Surgery 37 1021 1955
7. Freeman N E, Leeds F H, Elliott W C and Roland S I Thromboendarterectomy for hypertension due to renal artery occlusion J Am M Ass 156 1077 1954
8. Imber I and Clymer R H Jr Obstruction of the renal artery producing malignant hypertension N England J M 252 301 1955
9. Frelick R W and Shellenberger W D Abdominal aortic thrombosis producing a Goldblatt kidney Delaware M J 26 260 1954
10. Fisher E R and Corcoran A C Congenital coarctation of the abdominal aorta with resultant renal hypertension Arch Int M 89 943 1952

THE EXPERIMENTAL USE OF PERMANENT SHUNTS FOR RESECTION OF SEGMENTS OF THE THORACIC AORTA*

WILLIAM E NEVILLE AND GEORGE H A CLOWES JR

Successful resections of the descending aorta with periods of obstruction up to 77 minutes have been reported [4] However experiments and clinical data indicate that this is unsafe except in constrictive lesions with an accompanying adequate collateral circulation [5] On the other hand temporary occlusion of the ascending aorta cannot be tolerated for more than a few minutes without permanent brain damage or cardiac arrest To prevent these complications many ingenious methods have been devised among which are local or generalized refrigeration and the use of temporary shunts [1, 6, 9]

The purpose of this paper is to present our results with an experimental technique in which permanent shunts were used first for resection of the descending thoracic aorta and subsequently the arch

METHOD

Resection of the Descending Aorta Under nembutal anesthesia (60 mg per kg of body weight) dogs were placed in the right lateral position The chest was entered through the left fifth interspace aseptically The descending thoracic aorta was dissected from the left brachiocephalic artery to the diaphragm All of the intervening intercostal vessels were divided A modified Beck clamp was placed across the aorta just below the left brachiocephalic artery Following an adequate longitudinal incision in the vessel the previously prepared graft was sutured end to side to the aorta

*From the Department of Surgery Western Reserve Medical School and Cleveland City Hospital Cleveland Ohio The technical assistance of Mr Howard Peacock is greatly appreciated

Table 2 Aortic Arch Replacement

	GRAFT	LENGTH OF SURVIVAL	CAUSE OF DEATH	REMARKS
1	Nylon Taffeta	24 hours	Inadequate cerebral blood flow	
2	Nylon Taffeta	Died on table	Rupture of ascending aorta	
3	Nylon Taffeta	Died on table	Ventricular Fibrillation	
4	Nylon Taffeta	Died on table	Rupture of ascending aorta	
5	Nylon Taffeta	18 hours	Hemorrhage—graft intact	Given heparinized blood during procedure—bleeding from mediastinum
6	Nylon Taffeta	Died on table	Rupture of ascending aorta	
7	Nylon Taffeta	2 weeks		Heavy cord cut through the aorta
8	Chemstrand Nylon	Died on table	Hemorrhage from ascending aorta	
9	Chemstrand Nylon	7 weeks	Distemper	Graft intact—no thrombosis
10	Homologous artery	Died on table	Hemorrhage from ascending aorta	
11	Homologous artery	4 weeks	Massive pleural effusion	Suture lines intact—no thrombosis
12	Homologous artery	3 weeks	Hemorrhage	Heavy cord cut through the aorta
13	Homologous artery	1 week	Empyema	Suture lines intact no thrombosis
14	Homologous artery	5 days	Massive pleural effusion	
15	Homologous artery	Died on table	Hemorrhage from ascending aorta	
16	Homologous artery	Died on table	Hemorrhage from ascending aorta	
17	Homologous artery	Died on table	Hemorrhage from ascending aorta	
18	Homologous artery	Died on table	Hemorrhage from ascending aorta	

the outset straight nylon tubes with 2 arms corresponding to the brachiocephalic and left subclavian arteries were used in 7 instances for reconstruction of the arch Although satisfactory blood flow could be obtained through this shunt the acute angulation at the anastomosis on the ascending and descending aorta caused disruption of the ascending aorta in 6 animals One animal survived 2 weeks his demise having been caused by the heavy string eroding through the host aorta

In 2 animals pliable tubes of Chemstrand nylon were used The distal ends of the brachiocephalic arteries were sutured to the convex surface of the shunt Autopsy of 1 of these animals in a week demonstrated that the graft was intact with no evidence of thrombosis

Five dogs died during the operation as a result of hemorrhage from the ascending aorta when the homologous grafts preserved in alcohol were used In this group 4 dogs survived the procedure Although they succumbed as a result of complications in from 1 to 4 weeks all were active and exhibited

sutured end-to-side (Figs. 1 & 2). The anastomosis was performed in the manner previously described. After testing the suture line, the distal end of the graft was similarly anastomosed to the descending thoracic aorta. When adequate blood flow had been established through the shunt, the arms of the graft were sutured end-to-end to the divided brachiocephalic arteries (Figs. 3 & 4). To prevent irreversible brain damage, it is essential that division and anastomosis of these vessels be performed individually while the other brachiocephalic vessel is functioning. When satisfactory blood flow *has been established both cephalad and caudad, the intervening arch of the* aorta was either divided or ligated in several places with heavy string.

Table 1. Resection of the Descending Thoracic Aorta

	GRAFT	LENGTH OF SURVIVAL	CAUSE OF DEATH	REMARKS
1	Nylon Taffeta	5 weeks	Sacrificed	Suture lines intact, no thrombosis
2	Nylon Taffeta	4 weeks	Sacrificed	Suture lines intact, no thrombosis
3	Nylon Taffeta	6 weeks	Sacrificed	Suture lines intact, no thrombosis
4	Nylon Taffeta	24 hours	Rupture of anastomosis	
5	Nylon Taffeta	2 months	Sacrificed	Suture lines intact, no thrombosis
6	*Chemstrand Nylon*	*1 week*	*Sacrificed*	*Suture lines intact*, no thrombosis
7	Chemstrand Nylon	3 weeks	Sacrificed	Suture lines intact, no thrombosis
8	Chemstrand Nylon	Living (7 mos.)		
9	Chemstrand Nylon	5 weeks	Sacrificed	Suture lines intact, no thrombosis
10	Chemstrand Nylon	8 weeks	Sacrificed	Suture lines intact, no thrombosis

RESULTS

Resection of the descending aorta was performed on 10 dogs (Table 1). All of the animals survived the operation and none exhibited hind limb paralysis. In 6 animals tubes of nylon taffeta were used. These were sewn along the free edges and beveled at either end. It is significant that despite *some kinking due to angulation, only 1 dog died of disruption of the suture* line. The remainder were sacrificed. Seamless flexible tubes of nylon were used in the remaining 4 animals to reconstruct the aorta. Although it is technically more difficult to perform the anastomosis, the flexibility of the tube precludes kinking at the suture line. One of these animals is still living 7 months following operation.

In a control series of 5 animals the intervening aorta was resected between Potts clamps. Arterial continuity was established by intercalating a synthetic graft between the divided aorta. Even though the period of total aortic occlusion was less than 20 minutes, all of the animals developed hind limb paralysis.

Resection of the aortic arch was performed on 18 dogs (Table 2). At

graft and the aortic valve In several dogs a break in the vessel wall was clearly demonstrated where the toothed partial occlusion clamp had been placed It is apparent that dehiscence of the artery will not occur unless undue upward tension is put on the wall of the vessel It is significant that hemorrhage occurred after the entire arch had been replaced This confirms Carrell s observation in 1910 that in the dog the ascending aorta is extremely friable[3] In no instance did the clamp injure the descending aorta sufficiently to cause rupture

Although the mortality has been high in resection of the arch, several interesting observations have been made from the experiments An end to-side anastomosis can be performed in the descending aorta without rupture A similar permanent shunt can be performed between the ascending and descending aorta In contrast the friable ascending aorta is prone to post operative rupture due to trauma from the toothed clamp when upward pull or angulation is exerted following the brachiocephalic anastomosis It is also essential to individually divide and anastomose the brachiocephalic vessels to prevent permanent brain damage

SUMMARY AND CONCLUSIONS

1 Techniques are presented in which both the descending aorta and the arch can be resected using permanent end to side shunts

2 Nine of 10 animals survived resection of the descending aorta while 5 of 18 survived resection of the arch

3 Hemorrhage from the friable ascending aorta accounted for all of the deaths in the latter group

4 To prevent irreversible brain damage the brachiocephalic vessels have to be divided and sutured individually to the prosthetic arch

REFERENCES

1 Alley R D Sewell W H Formel P F Stranahan H Kausel H and Koth D R Experimental evolution of external shunts for bypassing the thoracic aorta Surgical Forum 1953 Philadelphia W B Saunders Co 1951 pp 85 90

2 Beattie E J Jr Adonasio W Keshishian J M and Blades B Refrigeration in experimental surgery of the aorta Surg Gyn Obst *96* 711 1953

3 Carrel A Report on the experimental surgery of the thoracic aorta and heart Ann Surg *52* 83 1910

4 Cooley Denton H and Debakey M E Resection of the thoracic aorta with replacement by homograft for aneurysms and constrictive lesions J Thorac Surg *29* 66 1955

5 Hufnagel C A and Gross R D Coarctation of the aorta experimental studies regarding its correction N England J M *233* 287 1945

6 Izant R J Hubay C A and Holden W D A nonsuture aortic shunt an experimental study Surgery *33* 233 1953

7 Johnson J Kirby C K and Lehr H B A method of maintaining adequate blood flow through the thoracic aorta while inserting an aorta graft to replace an aortic aneurysm Surgery *37* 54 1955

8 Mahorner H and Spencer R Arterial shunts a technique for replacing segments of large vessels Angiology *5* 294 1954

9 Pontius R G Brackman LeRoy Hardy F G Cooley D A and DeBakey M F The use of hypothermia in the prevention of paraplegia following temporary aortic occlusion Surgery *36* 33 1954

10 Satinsky V P Neptune W B and Alai J Experimental transplantation of the complete arch of the aorta Ann Surg *141* 38 1955

11 Stranahan H Alley R D Sewell W H and Kausel H W Aortic arch resection and grafting for aneurysm employing external shunt J Thorac Surg 29 54 1955

no evidence of cerebral damage or hindquarter paralysis. Postmortem examination revealed intact suture lines and no evidence of thrombosis.

DISCUSSION

In resection of aneurysms of the descending aorta the necessity of doubly cross-clamping the vessel and dividing the intervening intercostal arteries make spinal cord ischemia inevitable unless adequate collateral circulation is present.

Local refrigeration and generalized hypothermia have shown experimentally and clinically to give some protection against hindquarter paralysis. Until measures to prevent ventricular fibrillation and cardiac standstill in adults under hypothermia is found, endeavors to develop an adequate external shunt seem worthwhile. Temporary intra and extraluminal shunts have been used previously.[9] Although they have been effective in preventing paraplegia, their insertion and removal is time consuming and may be actually dangerous.

Our present studies indicate that a permanent shunt between the proximal and distal descending thoracic aorta is practical in animals. Although it might appear that the end-to-side anastomosis is more likely to disrupt because of tension, such has not been our experience. To prevent angulation of the graft at the suture line, certain conditions have to be met. In the first place, the graft should be beveled at either end in order for it to assume a normal position following resection of the intervening aorta. Secondly, the graft itself should be one that is unaffected by angulation. At the present, homografts and flexible Chemstrand nylon tubes seem best suited for this. In the beginning of this study, nylon taffeta grafts were used. At autopsy all of the grafts were found to be kinked. As has been demonstrated previously, this may result in eventual thrombosis of the graft.

In resection of the aortic arch, techniques have to be developed to insure adequate cerebral arterial flow since crossclamping the ascending aorta for only a few minutes usually results in death. The use of large bore temporary external shunts seems to offer a surgically feasible method for diverting blood about segments of the aorta.[11] This has certain disadvantages.

Our original success with the use of permanent shunts for the replacement of the descending aorta led us to investigate a similar technique for the aortic arch. There is no question that a permanent shunt can be employed between the ascending and descending aorta. Experimentally, the arch of the aorta can then be divided proximal to the brachiocephalic arteries with long term suvival. In these instances only lateral wall tension is put on the vessels. Complete replacement of the entire arch presents additional problems. Following anastomosis to the divided brachiocephalic arteries, the graft is pulled cephalad. Thus the strain is transferred mainly to the caudal ends of the suture lines on the ascending and descending aorta. Early experiments led us to believe that hemorrhage was due to disruption of the suture line from acute angulation of the nylon prosthesis. Initial pleasing results with the use of pliable homologous grafts preserved in alcohol seemed to justify this conclusion. Although these adapt themselves more readily to angulation, uncontrollable hemorrhage still occurred frequently from the ascending aorta. With further observations it became obvious that the hemorrhage occurred through the anterior wall of the aorta between the

to tolerate complete occlusion in one stage They were sometimes noted to limp for a day or two post occlusion but thereafter appeared normal Quite by accident one of the dogs was noted exercising several weeks post occlusion After several minutes he began to limp and could not be induced

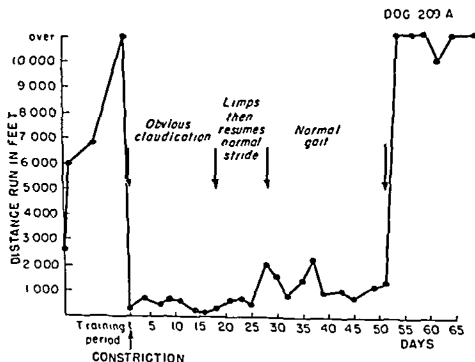


Fig 2

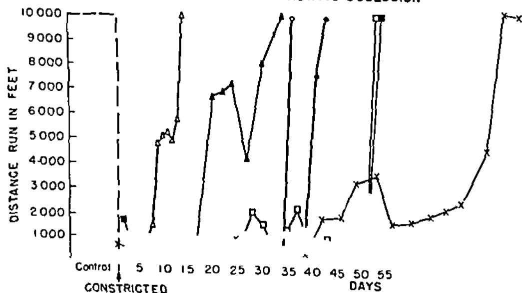


Fig 3

THE EXPERIMENTAL PRODUCTION OF INTERMITTENT CLAUDICATION*

Julius H. Jacobson II and Ferdinand F. McAllister

The study of intermittent claudication has been greatly hampered by the lack of a suitable experimental preparation. Human volunteers with claudication have been subjected to various exercise stresses and the effect of one or another agent assayed.[1] These investigations have been clouded by the extreme variability of the subjects and the obvious limitations imposed by the lack of an animal preparation. Controlled studies on the rate of collateral formation, the role of sympathectomy, and the value of concomitant vein ligation at the time of a sudden arterial occlusion have not been possible.

In devising a method for the controlled occlusion of larger blood vessels we found that we had an excellent animal preparation for the study of intermittent claudication. This consists in utilizing an inflatable rubber *cuff which occludes the terminal abdominal aorta in the dog.*

Figure 1A shows how the cuff* is put in place and used to control the flow of blood through the abdominal aorta. The circular portion is a rubber balloon with cloth tabs at either end which permit the balloon to be sewn together once it has encircled the vessel (Fig. 1B, C and D). Leading from this is a rubber catheter that is guided out retroperitoneally and has at its end a self sealing rubber diaphragm (see Fig. 1A for cross sectional detail) that is left beneath the skin. This latter feature allows the cuff to be inflated or deflated by a subcutaneous injection into it, thus eliminating the problem of sepsis that is present in any device allowed to protrude from the body for an extended period of time. In Figure 1E the cuff is shown in phantom with a needle being introduced subcutaneously into the self sealing rubber tip which is embedded beneath the skin. Using this device one can partially or completely occlude the aorta at any time subsequent to its installation by a simple hypodermic injection. This avoids laparotomy or even use of an anesthetic agent.

Dogs in which cuffs were placed around the abdominal aorta were found

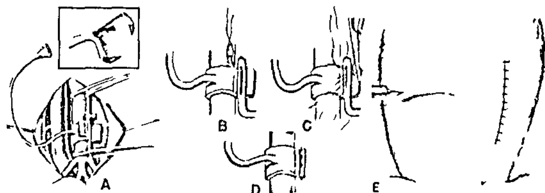


Fig. 1

*From the Departments of Surgery and Medicine, College of Physicians and Surgeons, Columbia University and the Presbyterian Hospital, New York, N. Y.

*Made for us by Davol Rubber Company, Providence, Rhode Island.

Figure 2 shows a typical record After complete aortic constriction running ability decreased immediately For the first 2 weeks the dog would run short distances but at the termination of each run was unable to bear its weight on the hind legs In later weeks limping would occur during the run and then disappear although the dog would refuse to run any considerable distance Finally the preconstriction distance would be abruptly resumed

Figure 3 illustrates the composite findings in 7 dogs The time required to return to apparent normalcy varied from 2 to 9 weeks This finding correlates well with x ray studies of collateral circulation development Figure 1A shows a normal aortogram Figure 1B the sparsity of collateral circulation at 2 weeks post occlusion and Figure 1C, the obvious collateral present at 6 weeks

Preliminary studies on the effect of bilateral lumbar sympathectomy in this preparation shows that there appears to be a more rapid return to normalcy Interestingly enough from the very beginning they run longer distances although noticeably limping, as though the circulatory insufficiency is as marked but the pain secondary to it is diminished

Concomitant occlusion of the aorta and vena cava appears to have a deleterious effect a number of these animals never resuming their preoperative levels

In conclusion this type of quantitative measurement of exercise tolerance in animals with circulatory insufficiency it seems to us is of more practical significance than information obtained by much more elaborate instrumentation It shows promise in evaluating many factors important in the therapy of peripheral vascular disease We realize fully that sudden arterial occlusion in a healthy dog is different than the same thing occurring in an arteriosclerotic vascular tree but believe the underlying factors of collateral formation role of spasm and response to drugs must be quite similar

REFERENCES

1 Boyd A M Ratcliffe A H Jepson R P and James G W H Intermittent claudication a clinical study J Bone Surg Brit Vol *31B* 325 1949

2 Kissin M Stein J J and Adleman R J A two step test of exercise tolerance in intermittent claudication Angiology *1* 141 1950

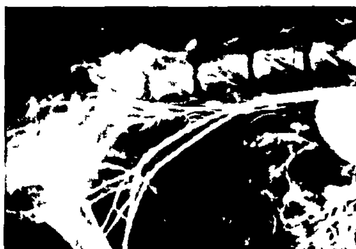


Fig 4a



Fig 4b



Fig 4c

to run further until he had rested for a few minutes After this he again ran but the same series of events recurred This mimicked exactly the syndrome of intermittent claudication as seen clinically Further study seemed indicated Accordingly a treadmill was secured and a number of dogs were trained to run on it for a distance of 2 miles at an arbitrary speed of 6 7 mph Only about 1 dog in 2 could be so trained A cuff was then placed in each of these dogs as just described Once the trauma of operation was over they were again run until they reached their preoperative level The aorta was then constricted completely

In 28 of these animals there was some degree of arterial oxygen unsaturation varying from 15 per cent to 91 per cent

RESULTS

There were 12 instances of severe histologic change in the lung all occurring in animals injected through the pulmonary artery Within 30 seconds after such an injection the affected lung showed a mottled appearance due to alternate patches of ischemia and congestion Lung biopsies were taken at intervals of 1 minute to 1 hour after injection Microscopic study of these specimens showed agglutinative thrombosis in capillaries (Fig 1) extending back into the arterioles and arteries in the more severe reactions The erythrocyte filled capillaries were dilated frequently to several times normal size and a pale staining fluid was often seen in the alveoli (Fig 2)

With biopsy of the lung delayed for 24 hours after such an injection the observed reaction was even more severe Grossly there were areas of congestion and atelectasis despite mechanical inflation Microscopically advanced pulmonary edema extensive congestion and atelectasis with vascular thrombosis were noted (Fig 3) Biopsy of the contralateral lung immediately after pulmonary artery injection revealed the normal histologic picture Examination of the contralateral lung after 24 hours showed mild to moderate vascular engorgement

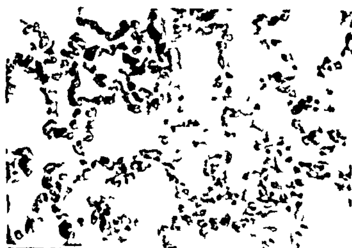


Fig 1 Biopsy of left lung of dog 15 minutes after injection of 70 per cent Urokon through left pulmonary artery Photomicrograph shows capillary engorgement indicative of moderate reaction X220



Fig 2 Pulmonary biopsy 20 minutes after ipsilateral pulmonary artery injection of 70 per cent Urokon Photomicrograph shows sickling and agglutinative thrombosis with fluid in alveoli X400

AN EXPERIMENTAL STUDY OF TOXIC FACTORS IN ANGIOCARDIOGRAPHY*

J. Gerard Mudd, Julio C. Wong, John P. Wyatt and
C. Rollins Hanlon

As a result of our experience with deaths due to angiocardiography in patients and in dogs, we have tried experimentally to determine the basis for such fatal reactions. In reviewing some deaths from angiocardiography in dogs with venous obstruction to one lung, it appeared that toxicity might be related to the intensity and duration of contact between the radiopaque material and the lung substance.

We studied this possibility by injecting standard doses of 70 per cent Urokon (sodium 3-acetylamino-2,4,6-triiodobenzoate) at 3 sites: (1) the jugular vein, (2) the outflow tract of the right ventricle, (3) one of the main pulmonary arteries. The concentration of radiopaque medium is directly related to the anatomical site of injection. Material injected in the jugular vein is diluted to a considerable degree by mixing with venous blood returning to the right auricle. Material injected in the right ventricular outflow tract undergoes much less dilution, but the amount and concentration delivered to one lung are less than with direct injection of the radiopaque material into a single pulmonary artery. This latter technique would be expected to initiate the greatest number of unfavorable reactions, and our studies tend to substantiate this hypothesis.

We also investigated the possibility that reduced oxygen saturation of the blood in a patient or experimental animal increases the likelihood or severity of reactions.

METHOD

Mongrel dogs were anesthetized with veterinary nembutal, and pulmonary oxygenation was maintained by an anesthesia machine delivering oxygen through an endotracheal tube. Varying percentages of blood oxygen saturation were achieved by inhalation of graduated mixtures of oxygen and nitrogen. Seventy per cent Urokon was given in a dosage of 1 cc. per kg. of body weight. The material was injected manually from a 20 cc. syringe through a #15 gauge needle into the jugular vein or through a #9 cardiac catheter in the case of the right ventricular outflow or pulmonary artery injections. The lung was observed at thoracotomy in many animals, and color photographs and roentgenograms were made together with periodic biopsies of the lung. The oxygen saturation of the arterial blood was determined from specimens drawn through an indwelling needle in the femoral artery.

Forty-one dogs were studied. Twenty-two were injected through the jugular vein, 5 through a cardiac catheter in the outflow tract of the right ventricle, and 14 through a catheter in the right or left pulmonary artery. With one exception, at least 2 separate injections were made in the animals injected through the jugular vein and right ventricular outflow tract. Five of these had 3 injections. Of the pulmonary artery injections only 5 of 14 were repeated.

*From the Departments of Medicine, Pathology and Surgery, St. Louis University, St. Louis, Missouri.

In 28 of these animals there was some degree of arterial oxygen unsaturation varying from 15 per cent to 91 per cent

RESULTS

There were 12 instances of severe histologic change in the lung all occurring in animals injected through the pulmonary artery Within 30 seconds after such an injection the affected lung showed a mottled appearance due to alternate patches of ischemia and congestion Lung biopsies were taken at intervals of 1 minute to 1 hour after injection Microscopic study of these specimens showed agglutinative thrombosis in capillaries (Fig 1) extending back into the arterioles and arteries in the more severe reactions The erythrocyte filled capillaries were dilated frequently to several times normal size and a pale staining fluid was often seen in the alveoli (Fig 2)

With biopsy of the lung delayed for 24 hours after such an injection the observed reaction was even more severe Grossly there were areas of congestion and atelectasis despite mechanical inflation Microscopically advanced pulmonary edema extensive congestion and atelectasis with vascular thrombosis were noted (Fig 3) Biopsy of the contralateral lung immediately after pulmonary artery injection revealed the normal histologic picture Examination of the contralateral lung after 24 hours showed mild to moderate vascular engorgement

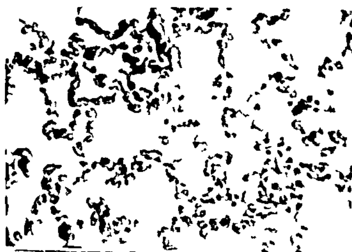


Fig 1 Biopsy of left lung of dog 15 minutes after injection of 70 per cent Urokon through left pulmonary artery Photomicrograph shows capillary engorgement indicative of moderate reaction X220



Fig 2 Pulmonary biopsy 20 minutes after ipsilateral pulmonary artery injection of 70 per cent Urokon Photomicrograph shows sickling and agglutinative thrombosis with fluid in alveoli X400



Fig 3 Pulmonary biopsy 24 hours after ipsilateral pulmonary artery injection of 70 per cent Urokon Photomicrograph shows extensive edema congestion and focal areas of vascular thrombosis X20

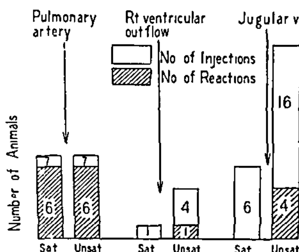


Fig 4 Graph showing number of pulmonary reaction in 41 animals subjected to Urokon injection Note high incidence of reaction in pulmonary artery injections There is inconclusive evidence for aggravation by arterial oxygen unsaturation



Fig 5 Pulmonary biopsy 15 minutes after a second injection of Urokon into the jugular vein Photomicrograph shows focal areas of mild capillary congestion X210

The listing of reactions according to site of injection is seen in Figure 4. There were 22 jugular vein injections with only 4 examples of pathologic change in the pulmonary parenchyma. These 4 occurred in the unsaturated group and were typified by mild to moderate focal areas of congestion as seen in Figure 5.

Of 5 animals injected through the right ventricular outflow tract, only 1 dog (in the unsaturated group) had a mild reaction.

Of 14 animals injected through the right or left pulmonary artery, only 2 failed to show pathologic evidence of damage. These 12 reactions occurred equally in the fully oxygen saturated and unsaturated groups.

DISCUSSION

It is possible that pulmonary damage in these animals results from hypertonicity of the contrast medium. The osmotic effect of 70 per cent Urokon is 7 times that of plasma. This may be directly injurious to the pulmonary vessels, as was demonstrated by Broman and Olsson[1] for cerebral vessels in studies on toxicity of contrast material in cerebral angiography.

It is assumed that the degree of damage from the injected material varies with concentration, dosage and duration of contact. Dosage was standardized and concentration depended only on variation in the site of injection. The duration of contact was prolonged in the damaged lungs, as judged by angiography and by persistence of Evans blue dye injected simultaneously in some animals.

Our results give evidence of direct damage to pulmonary tissue as indicated by pulmonary edema, congestion and thrombosis in both capillaries and larger vessels. This reaction is most severe with higher concentrations of the contrast medium such as occur with injection directly into a pulmonary artery. On the other hand, jugular injections, even when given 2 or 3 times, elicit relatively little reaction. Our data are insufficient at present to evaluate the part played by arterial unsaturation in these changes.

The relation of these experimental findings in dogs to toxic reactions in patients is uncertain. Our studies indicate that direct injection of contrast medium into the pulmonary artery of patients should be approached with considerable caution.

SUMMARY

In 41 dogs 70 per cent Urokon (sodium 3-acetylamine-2,4,6-triiodobenzoate) was injected at 3 sites: (1) the jugular vein, (2) the right ventricular outflow tract, and (3) the right or left pulmonary artery.

Significant gross and microscopic changes in the lungs were observed. These alterations were most severe with the injections into the pulmonary artery.

REFERENCE

1. Broman, T., and Olsson, O.: Tolerance of cerebral blood vessels to a constant medium of the diodrast group. Acta radiol., Stockh. 30: 326, 1948.

ANATOMICAL RESULTS OF ENDARTERECTOMY*

WILEY F. BARKER, JACK A. CANNON, LOUIS J. ZELDIS AND PERRY AH TYE

Two major types of operation are available for the treatment of arterial obstruction due to atherosclerosis (1) replacement by prosthesis and (2) reconstitution by the use of endarterectomy and repair of the patient's own vessel. Extensive reports have been presented that reveal the patterns of healing after the use of prostheses. There have been, however, few observations made on these patterns of healing after endarterectomy.

The following material attempts to answer the 4 questions that are most commonly asked about the anatomical and physiological results of endarterectomy.

1. Histological repair. What happens to the raw muscle tube that forms the reconstituted artery?

Material was available for study from 4 patients on whom endarterectomy was performed 5 to 90 days prior to death. (Two of these cases were from the personal series of W.F.B.) Figure 1 represents a section of a healed femoral artery 5 weeks after operation. The reconstructive processes differed so much in appearance in other vessels that experimental studies were



Fig. 1. Left. Microscopic section of distal femoral artery (human) 5 weeks after endarterectomy showing relatively normal media, a thickened fibrous subendothelial layer and a normal endothelium.
Fig. 2. Right. Microscopic section of dog artery 3 days after endarterectomy showing endothelium proliferating and extending over a latticework of fibrin.

*From the Department of Surgery, University of California Medical Center at Los Angeles and Wadsworth Hospital, Veterans Administration Center, Los Angeles, California.
This work has been supported by Grant Number H 1787 United States Public Health Service.

undertaken using the iliac arteries of dogs. Material was obtained after performing bilateral iliac endarterectomy in the dogs. Through a short incision in the distal iliac artery the intimal flap was elevated and the dissection of intima was carried proximally as dissection in a tunnel to simulate the closed technique recently described.[1] The vessel was closed after it was irrigated with dilute heparin solution. Systemic heparin was not continued. The specimens were fixed in 10 per cent neutral formalin. Stains used included hemotoxylin eosin, periodic acid Schiff, Verhoef, and a modified Wilder technique.

The narrow iliac artery (3 to 5 mm.) and the failure to use heparin resulted in thrombosis of most specimens and studies are therefore incomplete. Nevertheless certain preliminary observations appear to be of interest although exact application to the understanding of the healing of the human vessel cannot be made.

There is prompt and extensive proliferation of endothelium in uninjured portions of the artery at the margins of the stripped segments. Such proliferating endothelium may, within 18 hours, cover considerable areas of the stripped segment in which the internal elastic membrane has been left intact. With only superficial disruption of the media a thin layer of fibrin strands may, in favorable circumstances, form a delicate lattice which supports the ingrowth of a covering endothelium (Fig. 2). In either instance an essentially normal or only slightly thickened intimal layer may be reconstituted.

Observations thus far suggest that endothelium will not cover smooth muscle cells of exposed media directly. If fibrin is not first deposited reconstitution of an endothelial lining is apparently delayed until fibroblasts cover the media. Studies to examine the influence that the extent and duration of heparinization have upon the deposition and character of a fibrin layer are in progress.

In contrast to the prompt reparative reaction of endothelium the rather strictly limited range of response to injury on the part of the media is impressive. In segments of dog artery which have been stripped into the medial layer by tunneling rather than by incision of the wall, the vessel as long as 10 days later may show little evidence of healing. In fact the possibility is suggested that in such vessels the pattern of healing is greatly influenced by necrosis of portions of the remaining media which form portals of entry for granulation tissue originating in the adventitia. Fibroblasts and endothelial sprouts ultimately penetrate the necrotic wall and spread through and within it to form cleavage planes on which an intimal layer may develop. During this complex process the caliber of the vessel may be considerably diminished. Where the vessel has been stripped through an incision rather than by tunneling, granulation tissue from the adventitia is frequently seen to penetrate into the wall in the incised area and to spread out over the lumenal surface. There is some suggestive evidence that the ultimate differentiation of this granulation tissue may be affected by the presence or absence of segments of the internal elastic lamella beneath it.

2. Late structural integrity. How can the thin walled vessel withstand the arterial pressure?

Aneurysmal dilatation following endarterectomy has been *reported* by Kunlin. In the present series of approximately 100 cases only one real

aneurysm has been identified. In this patient hematoma and infection led to a ballooning of an endothelium lined space and rupture with fatal hemorrhage. Material from the femoral artery proximally reveals a well supported artery with no tendency to dilation (Fig. 1).

Several factors mitigate the risk of aneurysm formation. First is the fact that the wall, although thin, retains much of the original muscular coat with its resiliency. Second, considerable adventitial fibrosis occurs in the stripped vessel even in the abdomen where no structurally sound layers cover the endarterectomized segment. In the leg muscular planes in general enclose the artery and add extra support. Third, insofar as the lateral pressure on the wall is related to the diameter, an attempt has been made to narrow the lumen slightly when the vessel appears unduly thin walled. A smooth unchanging caliber is sought by performing arterial repair over an indwelling urethral catheter as a stent.

3. Late thrombosis. How soon can one anticipate thrombosis of the newly endarterectomized segment or occlusion by atheroma?

The experience of the authors has been that both arterial grafts and endarterectomies suffer their greatest risk from thrombosis in the initial hours or days after operation. Success depends upon an adequate perfusing pressure from the proximal tree and an adequate flow based upon freedom from distal obstruction. This obstruction might take the form of stenosis at the distal suture line in a graft or the distal extent of endarterectomy or the presence of obstructing atheroma beyond the scope of surgical repair. Careful technique, accurate suture attachment[3] and distal angiographs[4] have minimized the risk of thrombosis. Indeed in this series no late thrombosis of the endarterectomized segment has been encountered in those cases in which a definite pulse was clearly palpable postoperatively in follow up periods as long as 1 years. One patient had an incomplete main line flow re-established in 1950 because proximal and distal plaques were not removed. At subsequent reoperation in 1955 liquid pulsatile blood was present in the previously endarterectomized aortic segment although it was in continuity with only lumbar collaterals. There was no grossly apparent atherosclerosis in the original operative area.

4. Restoration of collaterals. What happens to the small branches that arise from an endarterectomized segment?



Fig. 3. Femoral angiograph made at the time of operation showing restoration of flow to a major muscular branch in midthigh.

de Takats[5] has mentioned the occurrence of necrosis of the sartorius muscle following replacement of the superficial femoral artery by a graft. A bypass graft, of course, should not destroy any previously unobstructed collateral branches. Completely satisfactory perfusion of all of the small muscular branches, however, might not be expected to occur unless the original pattern of flow was restored. Figure 3 illustrates postoperative angiographic evidence of such perfusion. It has been suggested that these small branches may be filling peripherally from a collateral network. At operation, however, it is common to find that after completion of the arterial repair bleeding occurs from the cut proximal ends of small muscular branches that had not bled when originally divided. Pulsation in small muscular branches may also be demonstrated after distal occlusion.

SUMMARY

An attempt is made to present some of the histological aspects of arterial repair after endarterectomy. Clinical observations indicate that aneurysm after endarterectomy is very uncommon. Late thrombosis or atherosclerotic obstruction of the segment operated upon should not be expected to occur in periods up to 3 years if the initial result is satisfactory. One of the advantages of endarterectomy is that it restores flow through the small arterial branches arising from the endarterectomized segment. The authors conclude that endarterectomy is an anatomically and physiologically sound technique that is useful in the reconstruction of obstructed major arteries.

REFERENCES

1. Cannon, J. A., and Barker, W. F. Successful management of obstructive femoral arteriosclerosis by endarterectomy. Surgery, *38*: 48-59, 1955.
2. Kunlin, J. Developement aneurysmatique apres thrombo-endarteriectomie de J. Cid dos Santos. Mem. acad. chir., Par., 55: 183-187, 1948.
3. Barker, W. F., and Cannon, J. A. An evaluation of endarterectomy. Arch. Surg., *66*: 488-494, 1953.
4. Barker, W. F. Distal operative angiography as an aid in endarterectomy. Surgery, *36*: 235-236, 1954.
5. de Takats, G. Revascularization of the arteriosclerotic extremity. Arch. Surg., *70*: 5-16, 1955.

Problems in Pulmonary Physiology and Pathology

HYPERVENTILATION IN THE TREATMENT OF CRUSHING INJURIES OF THE CHEST*

E. Trier Mörch, Edward L. Avery and D. W. Benson

A new method of treatment is presented for critically crushed chests using prolonged hyperventilation by means of a specially designed respirator giving a fixed volume for intermittent positive pressure endotracheal insufflation.

Severe crushing injuries of the chest wall carry a very high mortality mainly because of respiratory complications. Recently we have had the opportunity to treat 3 such patients by hyperventilation. The first case, which has been described in detail elsewhere, was especially dramatic. A 51 year old man was slowly rolled into an 8 inch space between a train and a steel furnace. He was admitted moribund in shock with bilateral

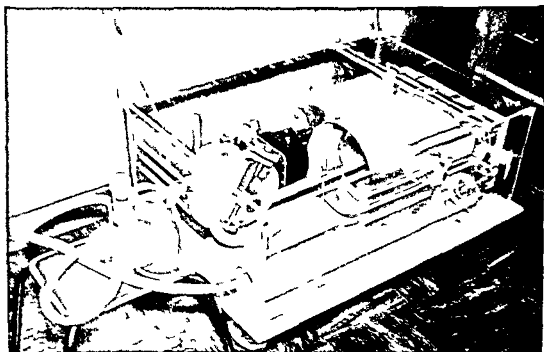


Fig. 1. The Mörch piston respirator.

*From the Department of Anesthesiology and Surgery, University of Chicago, and the Department of Surgery, Northwestern University Medical School. Aided by a grant (PHS#H 1502) from the National Heart Institute of the National Institute of Health, Public Health Service.

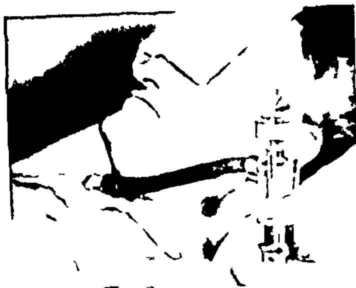


Fig 2 The respiratory air is blown in through a tracheotomy tube

fractures of most ribs costochondral separation a tension hemopneumothorax fractures of the sternum the clavicles and the pelvis crushing injuries of the liver and genito-urinary tract acute gastric dilation and paralytic ileus

The entire chest wall was so mutilated that external stabilization was insufficient The intermittent positive pressure piston respirator (Fig 1) developed by Dr E Trier Morch[1] at the University of Chicago was applied to a tracheotomy tube and adjusted to hyperventilate the patient (Figs 2 3) Within 4 hours the patient s condition improved from a semicomatose state of respiratory acidosis with a blood pH of 7 13 to a more alert state of mild passive respiratory alkalosis with a pH of 7 45 Mechanical hyperventilation was maintained for 30 days until the patient was able to breathe without paradoxical motions The patient was discharged from the hospital 51 days after the injury and has returned to his work

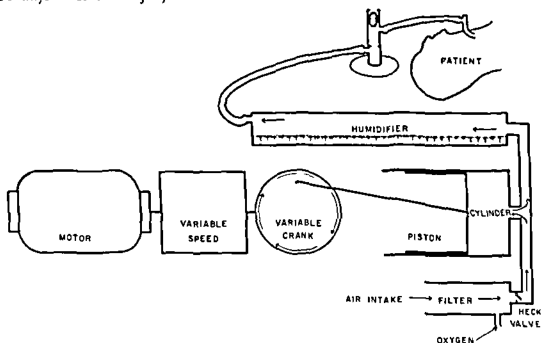


Fig 3 Morch piston respirator

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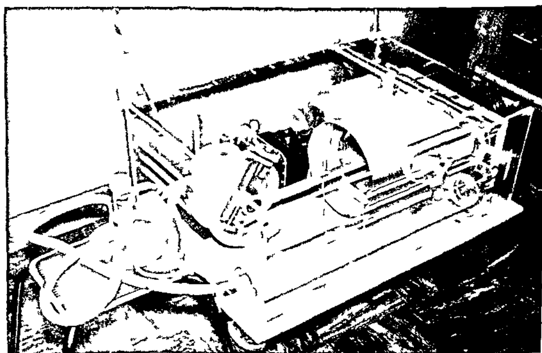


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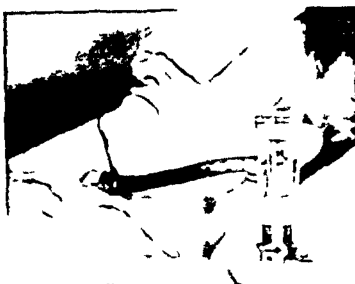


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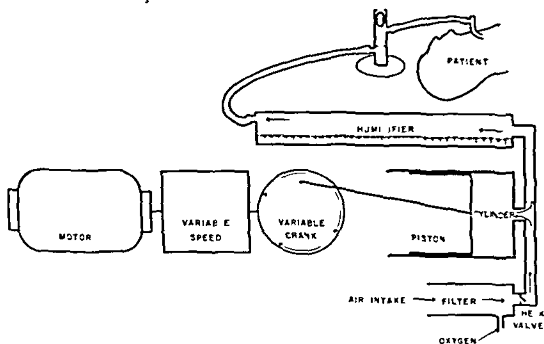


Fig 3 Mörch piston respirator

Problems in Pulmonary Physiology and Pathology

HYPERVENTILATION IN THE TREATMENT OF CRUSHING INJURIES OF THE CHEST*

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A new method of treatment is presented for critically crushed chests using prolonged hyperventilation by means of a specially designed respirator giving a fixed volume for intermittent positive pressure endotracheal insufflation

Severe crushing injuries of the chest wall carry a very high mortality mainly because of respiratory complications Recently we have had the opportunity to treat 3 such patients by hyperventilation The first case which has been described in detail elsewhere was especially dramatic. A 51 year old man was slowly rolled into an 8 inch space between a train and a steel furnace He was admitted moribund in shock with bilateral

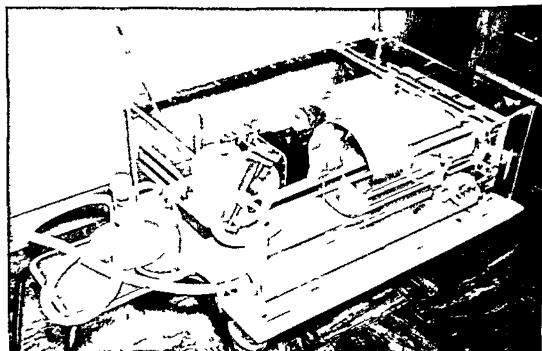


Fig 1 The Morch piston respirator

*From the Department of Anesthesiology and Surgery University of Chicago and the Department of Surgery Northwestern University Medical School Aided by a grant (PHS H 1502) from the National Heart Institute of the National Institute of Health Public Health Service

The manner in which hyperventilation counteracts paradoxical respiration and overriding of fractured ribs can be explained by a combination of two factors, a biochemical and a mechanical.

The *mechanical* factor is as follows.

During active inspiration the diaphragm pulls upward on the costal curvature and if this has lost its stiffness it will collapse. As the diaphragm descends, the intrathoracic pressure decreases to subatmospheric; the outside atmospheric pressure then pushes the soft parts of the thorax inward and paradoxical respiration and overriding results (Fig. 4). During active exhalation the reverse takes place. The intrathoracic pressure is now above atmospheric and all softened parts of the chest are pushed outward.

During passive inspiration the air is pushed into the lungs under pressure which exerts a gentle, evenly distributed push outward on all the softened parts, tending to bring them back into normal position. During passive exhalation the pressure drops toward room pressure so that the inside push gradually diminishes. In this way the alternating positive and subatmospheric pressure during active respiration has been replaced by a fluctuating positive pressure. The only rib motion is in their normal arcs while they passively ride on this cushion of air as the lungs gently and passively expand and contract. All paradoxical motion of the chest wall is stopped by this internal pneumatic stabilization.

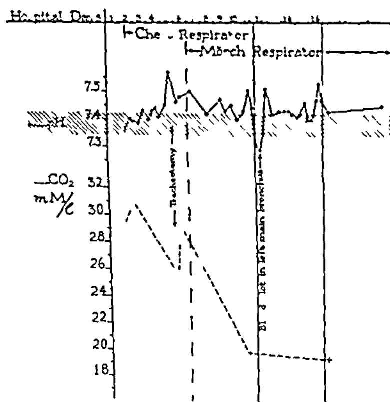
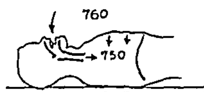
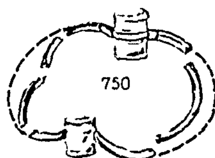


Fig 5. Prolonged hyperventilation and mild respiratory alkalosis

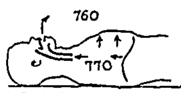
The *biochemical* factor is as follows.

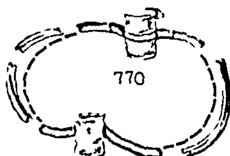
During hyperventilation carbon dioxide is washed out of the lungs. The CO_2 drops in the alveolar air, in the blood and in the respiratory center and active respiration ceases. The chest wall now follows the passive variations in pressure and paradoxical movements disappear.



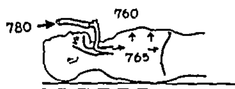


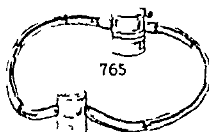
A ACTIVE INSPIRATION



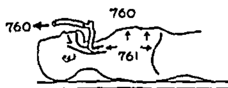


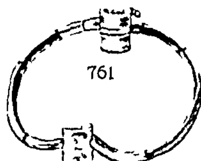
B ACTIVE EXPIRATION





C PASSIVE EXPIRATION
(mechanical)





D PASSIVE EXPIRATION
(mechanical)

Fig 1

AUTONOMIC CONTROL OF THE PULMONARY VASCULAR BED II DETECTION OF PULMONARY EDEMA WITH SODIUM^{24}*

MICHAEL C WEIDNER JR AUDLEY O BURFORD ROLLINS A DANIEL JR AND RUDOLPH A LIGHT

To our knowledge previous investigation of experimental pulmonary edema has been done by means of interval sacrifice and microscopic sections isolated perfusion experiments and open chest procedures In order to study this condition in the intact animal the use of a radioactive isotope to determine fluid shift into the lung has been utilized This permits analyses of the efficacy of various forms of therapy at any point in the development of edema and allows early detection of edema long before physical signs are apparent

METHOD

Adult mongrel dogs of varying ages weighing from 6.5 to 18 kg were used They were lightly anesthetized with intravenous veterinary nembutal 15 to 20 mg/kg

Fifteen microcuries of radioactive Sodium were given in an exposed femoral vein to each animal at the beginning of the experiment

In early experiments Geiger Muller tubes were used A Raytheon Model 20 Tube was placed over the femoral vessels to count the general circulation A polyethylene cylinder ¾ in diameter and 1" deep with a thin polyethylene end piece was placed in the chest wall against the pleura after resection of 1" of a rib usually the fifth on the left side adapted from the method of Jenkins Moyer *et al*[1] A Raytheon Model 105 end window counting tube was placed in this cylinder The G M tubes were fed through a differential count rate meter and the 2 tubes were balanced against each other Recording was done with an Esterline Angus DC recording milliammeter Model AW

In later experiments a scintillation counter with a sodium iodide crystal was placed against the chest wall and fed through a count rate meter (Fig 1)

One hundred mg/kg of 5 per cent alphanaphthol thiourea (ANTU)† in propylene glycol was injected slowly intravenously after the method of DuBois *et al*[2] and Drinker and Hardenbergh[3] Four hours later 50 cc/kg of 0.9 per cent sodium chloride was injected intravenously as this speeded up the onset of edema although alone it did not produce edema

Records were kept of pulse and respiratory rates and frequent examination of the chest with a stethoscope was made

After death or sacrifice all animals were autopsied noting the gross appearance of the lungs and sections were taken for microscopic examination In some animals serial biopsies of the lung were made during the

From the S R Light Laboratory for Surgical Research and the Department of Surgery Vanderbilt University School of Medicine Nashville Tennessee This work has been supported by a contract V1001M 4188 between the Veterans Administration and Vanderbilt University Medical School

†Purchased from Distillation Products Industries (Division of Eastman Kodak Co) Rochester N Y Remainder kindly donated by Dr C P Richter Johns Hopkins Hospital Baltimore Md

The main factor in overriding of the rib fragments is the active muscle pull. The respiratory apnea stops all active muscle motions and thus the overriding. The fragments are now supported by an air cushion.

When a patient with an injured chest is left to breathe on his own the ventilation is insufficient. Some degree of asphyxia will develop and result in excitement, restlessness and confusion. When mechanical hyperventilation is carried out, hypoxia disappears and with it the central stimulation. If sufficient carbon dioxide is blown out, a mild sedation has been found in most patients. The respiratory muscles can relax because their work is done for them and the result is that the patient rests and may even go to sleep.

The well known "hyperventilation syndrome" as seen in volunteers and in metabolic acidosis may lead to apnea, tetany, convulsions, unconsciousness and sometimes death. This however appears to be only true in active hyperventilation.

Circulatory changes caused by varying intratracheal pressures have been studied in man and in several experimental animals (dog, rabbit, guinea pig and bat). The systemic blood pressure and blood flow drops with increased intratracheal pressure. It is therefore important to keep the positive pressure phase short and the pause between the pressures long and with a low pressure. More important than the peak pressure is a minimal mean pressure.

SUMMARY AND CONCLUSION

A new method of treatment of patients with severely crushed chests is presented. Prolonged hyperventilation prevents

1. Paradoxical respiration.
2. Overriding of costal fragments.
3. It can replace all external means of chest wall stabilization.
4. Intermittent positive pressure respiration prevents hypoxia and hypercarbia.
5. Hyperventilation produces mild sedation.
6. Passive hyperventilation is safe.
7. It does not seriously alter the blood chemistry.
8. No later ill effects have been seen.
9. Circulation is not disturbed when the pressure variations in the trachea have the correct profile.

REFERENCES

1. Controlled respiration by means of special Automatic Machines as used in Sweden and Denmark. F. Trier Mørch, Ph.D., M.D. Proc. Royal Soc. Med. *40*: 39-43, 1947.

but no increase in counts. This discrepancy was due to failure in our differential counting rate meter as we were developing our tool.

Since it was apparent that fluid shifts from the circulation to the lung parenchyma and back again were being measured it appeared that differential counting of lung and peripheral circulation was no longer necessary. Since Na^{22} is a strong Gamma emitter, on the advice of Dr. C. W. Sheppard* a single scintillation counter against the chest wall was used.

Fifty five experiments were performed with the scintillation counter. Fatal pulmonary edema following ANTU and saline was produced in 31 dogs. There was an increase of counts averaging 1832 (range 800 to 6100) in all 31 (Figs. 2a and 2b and Table I).

Increase in rate and depth of respiration was usually preceded by an increase in counts though the length of time between the increase and the respiratory changes varied considerably. Rales and rhonchi and tracheal froth were invariably terminal events.

The control animal did not develop edema in the 12 hours it was observed; there was no change in counts and on sacrifice no gross or microscopic evidence of edema.

DISCUSSION

ANTU was used in these experiments only because it is fairly consistent in producing edema. Any other method for producing edema can be

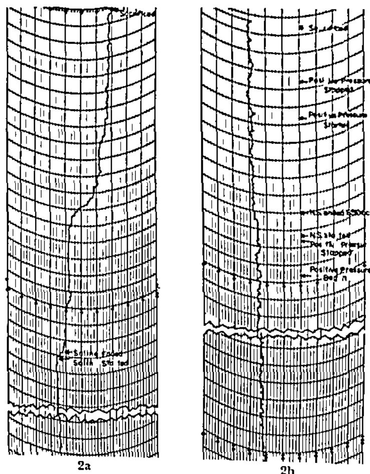


Fig. 2a. Development of pulmonary edema indicated by deflection of needle tracing to right. Each small division is equivalent to 400 counts.

Fig. 2b. Control on dog receiving no ANTU.

2a 2b

* Oak Ridge National Laboratories, Oak Ridge, Tenn.

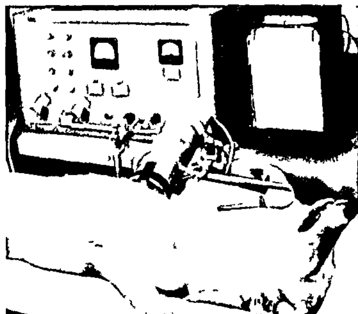


Fig 1 Photograph showing scintillation counter against the right chest wall the count rate meter and the Esterline Angus DC Recording Milliammeter

development of edema The animal was placed on positive pressure air respiration through an intratracheal tube while the chest was rapidly opened the biopsy made the lung repaired with a silk purse string suture and the chest rapidly closed The positive pressure was then discontinued until another biopsy was obtained

One control animal was anesthetized given Na^{2} saline and counted without receiving ANTU

RESULTS

Eighty five dogs were used Five per cent ANTU followed by intravenous saline produced fatal pulmonary edema in 53 of 85 dogs As Drinker[3] has found young animals and pregnant females were much more resistant to the effects of ANTU and it was here that the failures lay

The first 30 experiments were made with Geiger Muller tubes and differential counting between the lung and peripheral circulation Gross pulmonary edema was produced in 19 of 25 dogs to which ANTU and saline were given The onset of edema if it occurred varied from 1 to 6 hours after the saline injections an experience shared with Drinker and Hardenbergh[3] and DuBois *et al* In 11 of these 19 there was an increase in counts averaging 516 (range 292 to 800) This was such a small increase that for a long time there was considerable doubt that it was significant However by use of the serial biopsy technique and study of the microscopic sections conviction arose that actual measurements were being made of fluid lost from the circulation into the lungs Indeed it was possible to watch fluid move back into the circulation due to increased intrapulmonary pressure as the result of increased depth and rate of respiration When the respiration returned to normal an increase in counts would again occur This compensating mechanism could be seen to occur several times in occasional animals before a steady increase signalled that the animal was unable to compensate further This phenomenon undoubtedly represented the occurrence and disappearance of the interstitial phase of edema as described clinically by Altschule[4] In 6 animals there was edema

EXPERIMENTAL SILICOSIS: CHANGES IN THE DISEASE IN THE MONKEY PRODUCED BY ARTERIALIZING THE LUNG*

H. William Scott, Jr., C. Rollins Hanlon, Byron J. Olson,
George L. Lee and C. F. T. Mattern

Previous experiments[1-3] have shown that profound changes in induced pulmonary tuberculosis in animals are produced by various surgical alterations in the pulmonary circulation. Arterialization of the lung, whether accomplished by systemic-pulmonary anastomosis or by ligation of a pulmonary artery, results in severe exacerbation of the tuberculous process. In order to assess the effects of similar alterations in the pulmonary circulation on induced silicosis in monkeys, the present experiments were conducted.

METHOD

Thirty healthy nontuberculous *Macaca mulatta* monkeys were exposed to silica dust 6 hours daily for 133 days. They were separated into 4 groups: (1) nonoperated controls, (2) control thoracotomy, (3) division of one pulmonary artery, (4) anastomosis between innominate or left subclavian and a pulmonary artery. In the control thoracotomy and anastomosis groups some of the procedures were carried out before and others after the dust exposure. All of the pulmonary artery divisions were done before dust exposure.

Most of the animals were sacrificed between 1 and 2 years after removal from the dust chamber. The degree of pulmonary reaction was evaluated independently by several observers at autopsy. In addition to gross inspection and palpation, histologic preparations of entire lobes were made. These "giant slides" were also assayed independently by several observers for character and distribution of the fibrotic reaction. Correlation between appearance and basic reactivity of the lung was studied by chemical microanalysis of the serial sections immediately adjacent to those employed for histologic staining. The results of these chemical analyses and other studies designed to check on the uniform distribution of the silica throughout the lungs will form the basis for a subsequent report.

RESULTS

All of the animals showed substantial deposits of silica in both lungs. The reaction of the lungs was equal bilaterally in the nonoperated controls and in the controls which had simple thoracotomy. However, with division of the pulmonary artery to either side, the fibrotic reaction seemed more severe on the unoperated side. With anastomosis of an innominate or subclavian artery to a pulmonary artery, the unoperated side again showed a greater reaction in most instances. A more detailed consideration of the four groups follows.

Nonoperative Controls. There were 7 monkeys in this group. After the standard 5 months of exposure to silica dust, they were allowed to live

*From the Departments of Surgery of Vanderbilt University School of Medicine, Nashville, Tenn., and St. Louis University School of Medicine, St. Louis, Mo., and the Laboratory of Infectious Diseases of the National Institutes of Health, Bethesda, Maryland.

Table 1

TUBE	NO DOGS	GROSS PULMONARY EDEMA	NO DOGS WITH INCREASE IN COUNTS	AV INCREASE IN COUNTS
Geiger Müller	30	19	11	516 (292-800)
Scintillation	55	34	34	1832 (800-6400)

equally well measured by this simple and relatively inexpensive tool If edema is produced by hemodilution techniques more Na must be used so that its proportion does not change

Studies are now in progress on the effects of aminophyllin morphine positive pressure antifoaming agents etc in experimental pulmonary edema Also an attempt to gain additional data on the etiology of pulmonary edema is being made

SUMMARY AND CONCLUSIONS

1 A method of recording the development and course of experimental pulmonary edema utilizing Sodium as a tracer and a scintillation counter fed through a counting rate meter to an Esterline Angus recording milliammeter has been presented

2 It is believed that this tool will permit the evaluation of various forms of therapy as well as the etiology of pulmonary edema

REFERENCES

1 Jenkins M T Jones R F Wilson B and Moyer C A Congestive atelectasis—a complication of intravenous infusion of fluids Ann Surg *132* 327-347 1950

2 DuBois K P Holm L W and Doyle W L Studies on the mechanism of action of thiourea and related compounds J Pharm Exper Ther *87* 53-62 1946

3 Drinker C K and Hardenbergh E Acute effects upon the lungs of dogs of large intravenous doses of alpha naphthol thiourea (ANTU) Am J Physiol *156* 35-43 1949

4 Altschule M D *Acute pulmonary edema* New York Grune and Stratton 1954



Fig 3 Division of right pulmonary artery 3 months before 153 days of dust exposure Death 461 days after operation Extreme fibrosis in unoperated lung Photomicrograph x 6

Fig 4 End to-end anastomosis innominate to right pulmonary artery after 153 days in dust Died 287 days after operation Extreme fibrosis in unoperated lung Photomicrograph x 6

Pulmonary Artery Divisions In 6 animals the pulmonary artery was divided (3 right 3 left) 3 months before the standard exposure to silica These animals lived from 12 to 21 months after beginning exposure to dust 3 died and 3 were sacrificed The lung on the operated side resembled the lungs of control animals while the opposite lung showed an intense fibrotic response (Fig 3) The difference between the 2 lungs was unequivocal in all but 1 monkey in this animal the predominance on the unoperated side rested on a few large isolated areas of fibrosis

Anastomosis of Innominate or Subclavian to Pulmonary Artery This end to end anastomosis was carried out in 9 monkeys 6 with the innominate artery anastomosed to the right pulmonary artery 3 with subclavian to left pulmonary artery anastomosis In 3 animals the operations preceded exposure to silica by 3 months One of these monkeys showed a greater fibrotic reaction on the nonoperated side the others were similar bilaterally In 6 other monkeys operation was performed immediately after cessation of dust exposure Five of these showed greater reaction on the nonoperated side (Fig 4) in the sixth the fibrotic reaction was minimal bilaterally and predominance of the nonoperated side was questionable

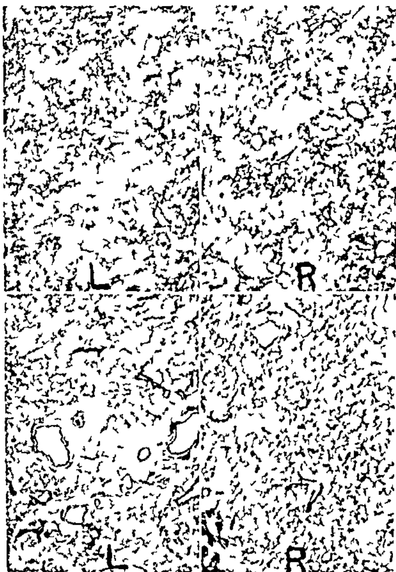


Fig. 1. Lung sections of nonoperated control monkey 153 days in dust chamber sacrificed 155 days after removal. Photomicrographs show emphysema and diffuse perivascular fibrosis equal bilaterally. × 6

Fig. 2. Lung sections of operated control monkey 153 days in dust followed by right thoracotomy. Death 205 days later. Photomicrographs show equal reaction bilaterally. × 6

for an additional 15 months. The fresh lungs at autopsy showed no significant difference, although there was some variation in the degree of fibrosis from animal to animal. Microscopic examination confirmed the impression gained from the gross material and demonstrated a fairly heavy accumulation of silica throughout both lungs with diffuse reticular perivascular fibrosis and mild emphysema (Fig. 1).

Controls with Thoracotomy. Eight monkeys underwent control thoracotomy (4 right, 4 left) with manipulation of the lung as in the ligation or anastomosis animals. Half of the monkeys were operated on 3 months prior to dust exposure while the remainder completed the standard 5 months in dust before operation. The mean interval between exposure to dust and sacrifice was 17 months, with variations from 6 to 20 months.

The 2 lungs showed a similar picture (Fig. 2) in all but one animal. The exception was 1 of 4 monkeys operated on before exposure to silica; the nonoperated side presented a significantly greater fibrotic response. In the other 7 animals the gross and histologic picture mimicked the nonoperated controls save for a mild fibrous reaction beneath the visceral pleura at the thoracotomy site.

cost Recently we have achieved hyperventilation by simply extending the patient's normal respiratory dead space with a rubber tube This results in an increased alveolar pCO_2 and subsequently an increased arterial pCO_2, which by central nervous system stimulation causes hyperventilation

METHOD AND RESULTS

1 Selection of a Standard Dead Space Tube Studies were performed on 9 normal personnel All lay supine with head supported on a pillow to simulate a patient's situation A nose clip was applied and the subject initially respired via a rubber mouthpiece attached to a double one way valve Inspiration was from room air and expiration through an electrically recording rapid gas flow meter from which minute volumes could be calculated Respiratory rate was noted and tidal volume computed Following a 10 minute control period 1 of several rubber tubes was inserted between the mouthpiece and the valve thereby adding respiratory dead space and a 10 minute test run was made

The average tidal of these 9 subjects was 591 cc and test runs demonstrated an average increase of 770 cc resulting in a stimulated tidal volume of 1360 cc if a 1000 cc dead space tube were used A 1000 cc tube made of black rubber with an internal diameter of 3 2 cm and a length of 125 cm (Fig 1) was therefore arbitrarily selected for further patient testing because it more than doubled the control tidal volume

2 Effect of Addition of 1000 cc Dead Space in Postoperative Patients Fourteen patients who underwent various surgical procedures were studied Control tidal volumes and stimulated tidal volumes with the addition of 1000 cc dead space were measured by the method outlined above Readings were taken preoperatively and on the first 3 postoperative days (Table I) In all 14 patients the addition of 1000 cc dead space approxi

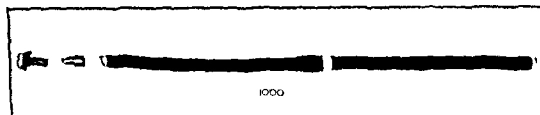


Fig 1 1000 cc rubber added dead space tube with rubber mouthpiece and metal connection

Table I Average of 14 Patients Studied Preoperatively and During the First 3 Postoperative Days

DAY	CC TIDAL VOLUME CONTROL	1000 CC ADDED DEAD SPACE	% INCREASE
Pre op	458	904	97
1st Post op	401	922	130
2nd Post op	410	925	126
3rd Post op	416	942	127

COMMENT

The reason for the relative decrease of the fibrosing reaction in the arterialized lung as compared with the unoperated side is uncertain Indeed the unoperated side is reacting more violently than normal possibly because it is receiving the full output of the right heart in contrast to the more limited but fully oxygenated blood supply to the operated lung This extreme fibrosis in the unoperated lung is a striking phenomenon of uncertain origin Neither lung in these experiments showed the typical picture of nodular silicosis seen in the chronic human disease No hypothesis concerning possible therapeutic significance of these results is advanced and further work is needed before this can be done

SUMMARY

In 15 monkeys with bilateral pulmonary deposits of silica dust arterialization of one lung by systemic pulmonary anastomosis or by ligation of a pulmonary artery resulted in a more severe fibrotic reaction in the non operated lung

There was no difference between the 2 lungs in 15 similar animals not operated on or submitted to control thoracotomy

REFERENCES

1 Hanlon C R Scott H W Jr and Olson B J Experimental tuberculosis I Effects of anastomosis between systemic and pulmonary arteries on tuberculosis in monkeys Surgery 28 209 221 1950

2 Scott H W Jr Hanlon C R and Olson B J Experimental tuberculosis II Effects of ligation of pulmonary arteries on tuberculosis in monkeys J Thorac Surg 20 761 773 1950

3 Olson B J Scott H W Jr Hanlon C R and Mattern C F T Experimental tuberculosis III Further observations on the effects of alteration of the pulmonary arterial circulation on tuberculosis in monkeys Am Rev Tuberc 65 48 63 1952

ADDITION OF DEAD SPACE TO PRODUCE HYPERVENTILATION FOR PROPHYLAXIS OF ATELECTASIS*

SEYMOUR I SCHWARTZ AND W ANDREW DALE

Atelectasis is recognized as a common and often dangerous complication occurring not only after surgery but also in other situations in which the tracheobronchial secretions are not properly cleared The prophylactic effect of hyperventilation induced by carbon dioxide rebreathing was initially demonstrated by Scott and Cutler[5] The use of a paper bag into which the patient rebreathes and accumulates CO_2 is a common method of stimulating greater tidal volumes However the small size of the ordinarily used paper bag as well as the constant leaks due to imperfect fitting about the face makes this method inadequate Rebreathing with 5 to 10 per cent CO_2 mixtures using a face contour mask effectively increases aeration of the lung but entails a bulky apparatus and increased

*From the Department of Surgery University of Rochester School of Medicine and Dentistry Rochester New York

cost. Recently we have achieved hyperventilation by simply extending the patient's normal respiratory dead space with a rubber tube. This results in an increased alveolar pCO_2 and subsequently an increased arterial pCO which by central nervous system stimulation causes hyperventilation.

METHOD AND RESULTS

1. Selection of a Standard Dead Space Tube. Studies were performed on 9 normal personnel. All lay supine with head supported on a pillow to simulate a patient's situation. A nose clip was applied and the subject initially respired via a rubber mouthpiece attached to a double one-way valve. Inspiration was from room air and expiration through an electrically recording rapid gas flow meter from which minute volumes could be calculated. Respiratory rate was noted and tidal volume computed. Following a 10 minute control period 1 of several rubber tubes was inserted between the mouthpiece and the valve, thereby adding respiratory dead space and a 10 minute test run was made.

The average tidal of these 9 subjects was 591 cc and test runs demonstrated an average increase of 770 cc. resulting in a stimulated tidal volume of 1360 cc. if a 1000 cc dead space tube were used. A 1000 cc tube made of black rubber with an internal diameter of 3.2 cm and a length of 125 cm (Fig 1) was therefore arbitrarily selected for further patient testing because it more than doubled the control tidal volume.

2. Effect of Addition of 1000 cc Dead Space in Postoperative Patients. Fourteen patients who underwent various surgical procedures were studied. Control tidal volumes and stimulated tidal volumes with the addition of 1000 cc dead space were measured by the method outlined above. Readings were taken preoperatively and on the first 3 postoperative days (Table 1). In all 14 patients the addition of 1000 cc dead space approxi-

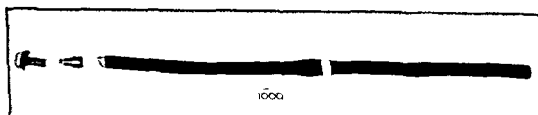


Fig 1. 1000 cc rubber added dead space tube with rubber mouthpiece and metal connection.

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1 Hanlon C R Scott H W Jr and Olson B J Experimental tuberculosis I Effects of anastomosis between systemic and pulmonary arteries on tuberculosis in monkeys Surgery *28* 209 221 1950
2 Scott H W Jr Hanlon C R and Olson B J Experimental tuberculosis II Effects of ligation of pulmonary arteries on tuberculosis in monkeys J Thorac Surg *20* 761 773 1950
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remained stable The alveolar pO_2 sharply diminished from a control value of 100.5 to 77.61 mm of Hg during the first 2 minutes of hyperventilation and over the remaining 8 minutes steadily and gradually rose to 88.03 mm of Hg

4 Effect of Introducing Oxygen Flow into 1000 cc Dead Space Tube The method outlined in part 3 was used A flow of 3 to 4 liters of 100 per cent oxygen per minute was introduced into the distal end of the rebreathing tube Tidal volume pCO_2 and pO_2 were studied in 3 subjects The average increases in tidal volume and pCO_2 of 700 cc and 7.2 mm of Hg respectively were similar to the changes noted in the 15 patients studied in part 3 However in all 3 patients the pO_2 was maintained above the control value of 100.5 mm of Hg (Fig 2) This indicates that while breathing through the 1000 cc. added dead space the elevation of pCO_2 is the critical factor effecting an increase in tidal volume

DISCUSSION

Hyperventilation with its attending alveolar distension has been considered an important factor in the prevention of atelectasis An increase in the alveolar pCO_2 raises the arterial pCO_2 which by central nervous system stimulation causes hyperventilation The addition of 1000 cc dead space into the respiratory passage causes a significant rise in alveolar pCO_2 and therefore effectively increases the respiratory tidal volume Duomarco y Diaz Romero[1] Harbord[2] and King[3] have described the use of added dead space to stimulate hyperventilation

Rebreathing the patient with a 1000 cc dead space tube is presently being used throughout the Strong Memorial Rochester Municipal Hospitals in a program directed toward the prevention of atelectasis A period of rebreathing for 5 minutes every 1 to 2 hours has been initiated and subjectively patients have found this method easier than either the paper bag or 5 per cent CO_2 delivered by means of a face contour mask While the nurse pinches the patient's nostrils shut the subject need only keep the rubber mouthpiece in his mouth and no additional cooperation is required As indicated in Fig 2 tidal volume is doubled by the end of 3 minutes of rebreathing equilibrates at this point and then remains stable The patient using the 5 minute therapeutic period is therefore actually rebreathing at a stimulated tidal volume of 2 times the control value for at least 2 minutes

The marked decrease in pO_2 which rises after 2 minutes (Fig 1) is ordinarily well tolerated by the patient In those individuals in whom anoxia is a concern a flow of 3 to 4 liters of 100 per cent oxygen per minute can be directed into the distal end of the rebreathing tube resulting in the maintenance of normal pO_2 while hyperventilation is accomplished This constitutes another distinct advantage over the paper bag method in which there is a constantly decreasing alveolar pO_2

SUMMARY

1 A method of inducing hyperventilation by means of the addition of 1000 cc dead space in the form of a rubber tube through which the patient breathes is outlined for use in the prevention of atelectasis

2 A parallel relationship between the increase in tidal volume and the

mately doubled the control volumes preoperatively and more than doubled the control values on the first 3 postoperative days. There was no significant alteration of the respiratory rate.

3. Relationship between Stimulated Tidal Volume, pCO_2 and pO_2. Tidal volumes, alveolar pCO_2 and alveolar pO_2 were measured during a 5 minute control period and 10 minutes of respiring through the 1000 cc. added dead space. Readings were made using the rapid gas flow meter combined with a Rahn continuous alveolar air sampler.[4] Fifteen subjects were selected from patients with normal pulmonary function studies. The results were uniform and Figure 2 represents the average of the 15 subjects during the control period and each of the 10 minutes of respiration through a 1000 cc. added dead space.

The pCO_2 and tidal volume curves closely parallel each other. The greatest increment in stimulated tidal volume occurred during the first 2 minutes during which time the tidal increased from a control value of 596 cc. to 1178 cc. During the third minute there was a smaller but nevertheless significant increase in tidal to 1331 cc. From the third through the tenth minute there was little change in the stimulated tidal volume. Similarly the greatest rise in alveolar pCO_2 from a control value of 39.41 to 44.17 mm. of Hg occurred during the first 2 minutes. A second increase from 44.17 to 47.20 mm. of Hg was noted between the second and fifth minutes and for the remainder of the 10 minutes the alveolar pCO_2

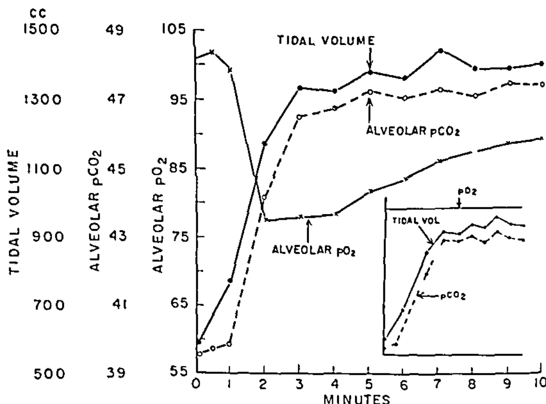


Fig. 2. Average of 15 normal subjects breathing room air through the 1000 cc. added dead space tube showing the relationship between tidal volume, alveolar pCO_2 and alveolar pO_2. Note the decrease in alveolar pO_2 when breathing room air (Main Graph) and the maintenance of normal alveolar pO_2 in 3 subjects when 3 to 4 liters of 100 per cent oxygen per minute were introduced into the distal end of the tube (Insert).

is 2½ hours at a flow rate of 500 to 3000 ml per minute The technique has been utilized clinically in the correction under direct vision of cardiac abnormalities both congenital and acquired

METHOD

Large (20 to 30 kg) mongrel dogs anesthetized with intravenous sodium pentothal (20 to 30 mg/kg) and heparinized (10 mg/kg) are subjected to a right thoracotomy in the fourth interspace Before the thoracotomy is completed the animal is sacrificed with an excess of sodium pentothal so that respiratory maintenance is not necessary After dividing the ribs above and below the thoracotomy incision for adequate exposure, the lungs and heart are removed *in toto* Care is taken to clamp and/or ligate all major vessels leading to or from the heart to prevent the entrance of air The trachea is divided at the apex of the mediastinum and cross-clamped to prevent aspiration into the lungs For clinical application the whole procedure is done under sterile conditions

After removal from the thoracic cavity the heart is easily and rapidly dissected free from the lungs and the pulmonary artery and trachea are cannulated The pulmonary veins are cut across at their entrance into the left atrium and are not cannulated Six per cent dextran* is then perfused through the isolated dog lungs at pulmonary artery perfusion pressures of 5 to 10 mm Hg and a flow of 100 to 300 ml per minute until the lungs are white and the dextran perfusate clear usually requiring 1500 to 2500 ml of dextran

METHOD

The clinical extracorporeal system is pictured in Figure 1 The accessory venous pick up the bottle depicted in the upper left hand corner serves two purposes (1) in the case of the temporary occlusion of the venae cavae catheter by collapse of the vessel about the aspirating holes admission of blood from the bottle serves to break the suction and (2) from these bottles the blood replacement required by the cardiotomy loss is accomplished

The pump† can deliver constant volumes against a large range of resistances Wide pulse pressures (0 to 150 mm Hg) were noted with this system due to the characteristics of the pumps and the relative rigidity of the plastic tubing‡ A depulsator consisting of a length of ⅞ inch penrose tubing housed in an air tight plastic cylinder, is interposed between the pump and the lung and serves to protect the lung against high ejection pressures A pressure transducer is used to record the pulmonary artery perfusion pressure The pulmonary artery is cannulated carefully to prevent undue tension or kinking The lungs are insufflated through an endotracheal cannula by means of an intermittent positive pressure respirator utilizing 100 per cent oxygen at pressures of 16 to 20 cm of water and a rate of 12 to 16 times per minute Expiratory resistance is kept minimal by means of a large bore expiratory outlet

Blood courses through the isolated dog lung emerging from the open pulmonary veins and is collected by the methacrylate lung chamber From

*Plavolex 6 per cent dextran in saline generously donated by Wyeth Laboratories Inc Philadelphia Pennsylvania

†Sigmamotor pump Model T6S Sigmamotor Corporation Middleport New York

‡Mayon plastic tubing Mayon Plastics Corporation Minneapolis Minnesota

alveolar pCO_2 with a concomitant decrease in pO is demonstrated This decrease in pO_2 can be prevented by directing a flow of oxygen into the distal end of the rubber tube

REFERENCES

1 Duomacio J y Diaz Romero C La prolongacion de espacio muerto respiratorio como medio practico de hiperventilacion pulmonar Arch urug med *10* 599 1937
2 Harbord R P Auto inhalation carbon dioxide therapy Brit J Anaesth *17* 13 1939
3 King D S Postoperative pulmonary complications II Carbon dioxide as a preventive in a controlled series J Am M Ass *100* 21 1933
4 Rahn H Mohney J Otis A B and Fenn W O A method for the continuous analysis of alveolar air J Aviat M *17* 173 1946
5 Scott W J M and Cutler E C Postoperative massive atelectasis The effect of hyper ventilation with carbon dioxide J Am M Ass *90* 1759 1928

STUDIES ON PERFUSION OF HUMAN BLOOD THROUGH THE ISOLATED DOG LUNG*

NORMAN WILLIAM CRISP JR GILBERT S CAMPBELL AND E B BROWN, JR

Isolated autologous and homologous canine lungs and lung lobes have been used in the physiologic research laboratory for many years Starling and Verney[1] perfused the isolated dog kidney using a modification of their heart lung preparation and maintained an *in vitro* renal blood flow approaching the calculated *in vivo* requirements Hemingway demonstrated the vasoconstrictor activity present in shed blood and that the isolated lung will detoxify as well as oxygenate the perfusing defibrinated blood

The search for suitable extracorporeal oxygenating systems for clinical use in direct vision intracardiac surgery has stimulated new research in the field of artificial oxygenators and a reinvestigation of the biological oxygenator in many laboratories Autologous and homologous isolated lungs and lung lobes together with extracorporeal pumping systems have been utilized successfully in several laboratories to maintain experimental animals during total cardiac bypass[3 4 5]

However the use of isolated heterologous lungs in such an oxygenator system has not met with comparable success Wesolowski Fisher and Welch[3] reported the rapid development of extensive congestion and pulmonary edema upon perfusion of the dog lung with human blood and include a photomicrograph showing the presence of severe pulmonary edema con gestion and intrabronchial hemorrhage after perfusion with human blood for 20 minutes Mustard and Chute[6] utilized monkey lungs to maintain infants during extensive intracardiac procedures

Utilizing the technique and precautions to be presented in this paper human blood has been perfused through the isolated dog lung for as long

*From the Department of Surgery and Physiology University of Minnesota Medical School Minneapolis Minnesota This investigation was supported by a grant from the U S Public Health Service (H 1784) and the Graduate School University of Minnesota

Figure 2A is a photomicrograph of a normal dog lung (magnification 510X) and shows the dog red blood cells in the capillaries and the normal architecture of the alveoli Figure 2B is a photomicrograph (magnification 510X) of the same lung after perfusion of 2000 ml of 6 per cent dextran at a pulmonary artery perfusion pressure of 5 to 12 mm Hg and a flow of 200 to 300 ml per minute Note the absence of red blood cells open capillaries and unaltered alveoli Figure 2C is a photomicrograph (magnification 570X) of the same isolated dog lung after 30 minutes of perfusion with A Rh positive human blood at a flow of 500 ml per minute and a pulmonary artery perfusion pressure of 25 mm Hg

DISCUSSION

There appears to be a direct correlation between the success in removing dog blood from the isolated dog lung by dextran perfusion and its subsequent ability to tolerate the perfusion of human blood without edema formation In some instances human blood has been perfused through the isolated dog lung at a flow of 3000 ml per minute and a pulmonary artery perfusion pressure of 16 mm Hg The lung provides an area for blood gas contact which is difficult to simulate in artificial systems and in addition to its function as an organ for rapid gaseous exchange the lung acts as a filter for abnormal blood constituents

SUMMARY

Human blood has been perfused through the isolated dog lung for as long as 2½ hours at a flow rate of 500 to 3000 ml per minute In order to prevent or minimize the development of pulmonary edema the following factors are deemed important (1) heparinization of the dog prior to removal of its lungs (2) open pulmonary veins (3) depulsation of the pulmonary artery limb of the extracorporeal perfusion circuit (4) ventilation of the isolated lung with a pressure of 16 to 20 cm of water avoiding expiratory resistance and (5) perfusion of large amounts of dextran through the lungs at a low pulmonary artery perfusion pressure (mean pressure less than 15 mm Hg) in order to rid the isolated dog lung of the heparinized dog blood



Fig 2 (A—Left) Normal dog lung (B—Center) Dog lung after dextran perfusion (C—Right) Dog lung after perfusion with human blood for 30 minutes

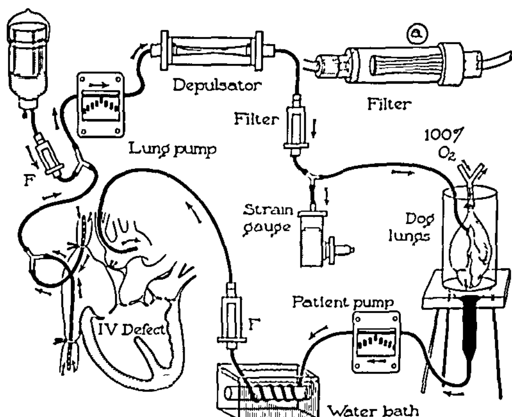


Fig. 1. The biological oxygenator is used clinically for direct vision intracardiac surgery.

this collecting reservoir oxygenated blood is returned by a separate pump through the water bath (11 to 12°C) and filter to the patient through the subclavian artery catheter. The filters in the system are loose meshed nylon filters housed in methacrylate chambers‡. Attention is called to the all plastic system. The Y connectors and pump fittings are methacrylate and the tubing is currently 3/16 inch internal diameter, 1/16 inch wall plastic hose. Exceptions are the short lengths of rubber tubing through the pump-heads and the penrose tubing in the depulsator.

For use in the laboratory substitution of a reservoir for the patient's heart or connecting the 2 separate limbs at this point allows recirculation of human blood through the isolated dog lung. The blood becomes rapidly oxygenated but no attempt has been made to introduce a deoxygenation device into the laboratory recirculation apparatus.

RESULTS

Isolated dog lungs previously cleared of dog blood by dextran perfusion have been perfused by recirculation of 500 to 1500 ml. aliquots of human blood for periods up to 2½ hours at flows up to 3000 ml. per minute and have remained edema free. These flows have been accomplished with pulmonary artery perfusion pressures of less than 25 mm. Hg. Bacteriologic studies made on banked human blood before and after perfusion through the isolated dog lung under aseptic conditions revealed no contamination. Platelet counts and white blood cell counts upon the same blood revealed a reduction of approximately 1/3 in these values.

‡Blood filter N 97 Baxter Laboratories Inc. Morton Grove, Illinois.

ance gauge or Statham strain gauge each calibrated against a mercury manometer Base line was set at the level of the board on which the dog lay

Five dogs survived with both defects the defects were closed between 31 and 34 months after creation and lung biopsies were obtained at this time Cardiac catheterization was performed again in the period after closure Five dogs survived with auricular defect alone

All dogs have ultimately been sacrificed their organs examined and histological studies made of the lungs

RESULTS

Systolic pressures in the pulmonary artery prior to operation did not exceed 30 mm Hg in any animal Average pressure in 12 dogs was 22/9 with an average mean pressure of 14 mm Hg Fifteen dogs survived the operative procedures and at least one postoperative cardiac catheterization In only one instance was the pulmonary systolic pressure less than 30 mm Hg the highest early pressure (first 14 days after operation) being 52/24 with a mean of 29 mm Hg In 3 dogs (dogs 13 19 20) pulmonary arterial pressure was unexplainably more than doubled on one or two occasions 2½ to 8 months after operation and later found to be nearly normal Except for ascites in 1 of these animals which later cleared no reason for the temporary elevation could be found In 1 of these 3 dogs the defect was found at autopsy to have closed spontaneously

Five dogs with an auricular septal defect alone and 5 with this defect plus an artificial ductus survived repeated catheterizations over a 32 to 44 months period after operation In no dog with an auricular defect alone was pulmonary hypertension with systolic pressure higher than 40 mm Hg either progressive or maintained (Figs 1 and 2 Table 1)

In 2 of the 3 animals (dogs 8 and 15) with an auricular defect and an artificial ductus which remained patent pulmonary hypertension was main

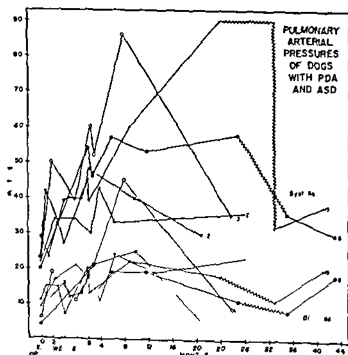


Fig 1 Pulmonary arterial pressures of dogs with artificial ductus and atrial septal defect Higher underlines represent systolic pressure lower narrow lines represent diastolic

REFERENCES

1 Starling E H and Verney F B The secretion of urine as studied on the isolated kidney Proc R Soc Ser B Biol Sc Lond *97* 321 363 1925
2 Hemingway A Some observations on perfusion of isolated kidney by pump J Physiol Lond *71* 201 213 1931
3 Wesolowski S A Fisher J H and Welch C S Heart lung bypass using pumps and isolated homologous lungs Surg Gyn Obst *95* 762 771 1952
4 Mustard W T and Chute A L Experimental intracardiac surgery with extracorporeal circulation Surgery *30* 684 688 1951
5 *Campbell G S Crisp N W Jr and Brown E B Jr Maintenance of respiratory* function with isolated lung lobes during cardiac inflow occlusion Proc Soc Exp Biol N Y *89* 390 393 1955
6 *Mustard W T and Chute A L A surgical approach to transposition of the great* vessels with extracorporeal circuit Surgery *36* 39 51 1954

EXPERIMENTAL PULMONARY HYPERTENSION WITH INCREASED PULMONARY BLOOD FLOW*

R BEVERLEY LYNN AND HENRY T BAHNSON

Pulmonary hypertension which is seen in many patients with heart disease may be a serious handicap to treatment of an otherwise remediable cardiac defect The cause of the elevated pulmonary blood pressure is not known but most studies indicate some interrelation of increased pulmonary blood flow or pulmonary outflow obstruction and pulmonary vascular lesions This report deals with an attempt to produce pulmonary hypertension by increasing pulmonary blood flow Flow was increased by creation of an auricular septal defect and an artificial ductus arteriosus two defects which often are associated with pulmonary hypertension when together in the same patient although either alone usually occurs without pulmonary hypertension The defects were closed after hypertension developed

METHOD

Twenty unselected young adult mongrel dogs were used Under ether anesthesia as large an auricular septal defect as possible was made by the method described by Hanlon and Blalock [1] In over half the dogs during the same procedure, the end of the brachiocephalic artery was anastomosed to the side of the right pulmonary artery In several instances this systemic pulmonary anastomosis was *done at a later date on the left side* the left subclavian artery being used In two 6 weeks old puppies an auricular septal defect was created

Cardiac catheterization was performed under light nembutal anesthesia prior to operation in all but 5 dogs and at intervals up to 11 months after operation in surviving animals Blood was drawn for determination of oxygen saturation on the Beckman D U spectrophotometer and O_2 capacity on the Van Slyke manometric apparatus Pulmonary pressure was measured with a Sanborn recorder and Sanborn electromanometer, Hathaway induct

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32 months in these animals. Pulmonary arterial pressure was normal when measured within the following month.

Lung biopsies and autopsy specimens showed no significant pulmonary vascular lesions. In 3 animals, in 2 of which both defects were made, the defects were found to have closed at autopsy. In all others defects were patent.

Attempts to estimate blood flows with the Fick principle from blood samples were dismally unsuccessful. The presence of shunts in anesthetized dogs made samples erratic and unreasonable.

DISCUSSION

This study was undertaken to determine if increased pulmonary blood flow causes increased pulmonary arterial pressure. Flow may be increased several fold for short periods in the normal human being without causing hypertension,[2] but pulmonary hypertension is commonly seen clinically in patients with defects which are normally associated with increased pulmonary blood flow, such as patent ductus arteriosus, ventricular septal defect or auricular septal defect.[3] In such instances histological changes are often present in pulmonary arterioles.[4] The question has remained unanswered, however, whether in these patients pulmonary hypertension results from long continued increased pulmonary blood flow or whether the condition represents a persistence of the fetal condition in which pulmonary pressure approximates systemic.

We interpret the present experimental study to show the following: 1. Pulmonary artery pressure rises slightly following the operation described. That this may be a non-specific rise associated with thoracotomy alone is suggested by its persistence even when the defects close spontaneously. 2. Auricular septal defect alone is not associated with development of pulmonary hypertension over a 3 year period even when the defect is created in young puppies. This defect does not necessarily cause an increased pulmonary blood flow.[3] 3. A striking elevation in pulmonary artery pressure does occur in some dogs with a combination of ductus and auricular defect. This appears within 2 to 8 months and can be reverted to normal within 1 month by closure of the ductus and closure, even though incomplete, of the auricular defect.

We were concerned principally with pressure changes. Several investigators have approached the problem by investigation of histological changes in pulmonary vessels. Levy and Blalock[5] performed the original Blalock operation in dogs with a purpose somewhat similar to ours but found no pathological lesions in the pulmonary arteries after about 6 months. Muller and associates[6] were able to produce histological changes in pulmonary arteries similar to those often encountered clinically with pulmonary hypertension by anastomosing the end of the left pulmonary artery to the aorta. Pulmonary hypertension was present when measured at operation and presumably persisted. A similar approach to the problem of pulmonary vascular lesions has been reported by Ferguson and associates.[7] In these experiments large end to end systemic pulmonary shunts were required to produce significant pulmonary vascular lesions. Such pulmonary vascular lesions seem related to increased pulmonary pressure rather than to increased blood flow.[8] Our studies were directed at the cause of the increased pressure

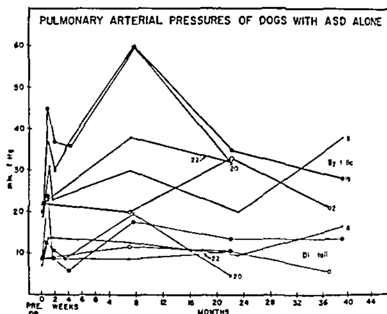


Fig 2 Pulmonary arterial pressures of dogs with atrial septal defect alone Higher underlines represent systolic pressure lower narrow lines represent *diastolic*

Table I

DOG NO	OPERATION	DURATION OBSERVATION MONTHS	REMARKS
8	ASD PDA	43	Defects closed surgically at 34 months Pulmonary pressure *36/8 one month later*
2	ASD PDA	34	ASD closed spontaneously
12	ASD PDA	43	Both? defects closed spontaneously
13	ASD PDA	32	Died following closure 2x3 cm ASD Ductus patent
15	ASD PDA	41	Defects closed surgically at 32 months Pulmonary pressure 32/11 one month later
18	ASD	39	10x12 mm ASD at autopsy
19	ASD	39	Defects closed spontaneously
20	ASD	40	ASD 40 mm in diameter at autopsy
21	ASD	37	25 mm ASD at autopsy Defect created at 6 weeks age
22	ASD	39	15 mm ASD at autopsy Defect created at 6 weeks age

tained on at least 2 occasions This was above 50 mm Hg in dog 8 and above 60 in dog 15 Dog 8 had no cardiac catheterization until 2 months after operation when pulmonary arterial pressure was 54/19 Pressures in dog 15 ranged between 27 and 45 systolic before it rose to 60 mm Hg at 9 months and to 90 mm Hg at 24 months The brachiocephalic artery was ligated and the auricular defect almost but not completely closed at 31 and

Clinical and Experimental Studies Relating to the Stomach, Small Bowel and Colon

INTRODUCTION

CLARENCE DENNIS

The observation of Wangensteen and his associates that peptic esophagitis is due to reflux of gastric juice into the lower esophagus has been confirmed by several previous presentations at the Surgical Forum. Merendino has indicated that the most effective measure to prevent such reflux is the insertion of a 12 cm. segment of jejunum between the terminal esophagus and remaining gastric pouch. In search of a technique less complicated than this, Girvin and Merendino this year report that utilization of combined complete vagotomy and pyloroplasty constitute a satisfactory procedure which has stood the test of histamine beeswax stimulation in the experimental animal.

The work of Everson several years ago indicated clearly that following total gastrectomy the metabolic maintenance of the subject is best served by retaining the duodenum in continuity. This work has been corroborated by many since that time and is further corroborated in this year's Forum by Binkley, Gardner, and McCorkle.

Berman introduced the utilization of a plastic prosthesis to replace resected segments of esophagus in the management of cancer of the esophagus several years ago. This year his technique appears to have been considerably improved, and it has been possible to perform such substitutions in a series of 40 dogs without leak in any.

Through the work of Dragstedt, and of Porter, French and associates in the last few years, it has become known that hypoglycemia in monkeys is followed by an increase in gastric acid occurring 2 to 3 hours after administration of insulin, even though complete division of the vagus nerves has been accomplished, and that this increase is mediated through the adrenal cortex and the pituitary. Thomas, Johnson and his associates this year have shown very nicely that the pituitary stalk apparently represents one link in the chain of responses bringing about this end result.

The mystery of Curling's ulcers has been investigated by Hummel and his associates who find an increase in urinary pepsinogen levels in patients with severe burns. The Curling's ulcers, however, occurred in the most severely burned patients, in which group the rise in urinary pepsinogen was somewhat less than that in the moderately severely burned individuals in whom Curling's ulcers did not develop. Further studies are indicated to determine the meaning of this discrepancy.

which often but not invariably is associated with increased pulmonary blood flow

These observations suggest that pulmonary hypertension produced by an increased pulmonary blood flow is not necessarily associated with organic changes in the pulmonary arterioles and that the condition is reversible by restoration of blood flow towards normal

SUMMARY AND CONCLUSION

Pulmonary hypertension can be produced in the adult dog by increasing pulmonary blood flow This has been accomplished by creation of an interauricular septal defect and an artificial ductus arteriosus The hypertension so produced is persistent but can be relieved by interruption of the ductus and closure of the auricular defect

No organic cause of the pulmonary hypertension was evident in lung biopsies which showed normal vessels

REFERENCES

1 Hanlon C R and Blalock A Complete transposition of the aorta and the pulmonary artery Experimental observations on venous shunts as corrective procedures Ann Surg *127* 385 397 1948
2 Slonim N Ravin A Balchum O J., and Dressler S H The effect of mild exercise in the supine position on the pulmonary arterial pressure of five normal human subjects J Clin Invest *33* 1022 1030 1954
3 Bahnson H T and Otis A B Physiological considerations of cardiovascular surgery Physiol Rev *35* 363 380 1955
4 Edwards J E Pathologic considerations in adjustments between the systemic and pulmonary circulations Henry Ford Hospital International Symposium on Cardiovascular Surgery Philadelphia W B Saunders Co., 1955 pp 100 117
5 Levy S E and Blalock A Experimental observations on the effects of connecting by suture the left main pulmonary artery to the systemic circulation J Thorac Surg *8* 525 530 1939
6 Muller W H Dammann F Jr and Head W H Changes in the pulmonary vessels produced by experimental pulmonary hypertension Surgery *34* 363 375 1953
7 Ferguson D J and Varco R L The relation of blood pressure and flow to the development and regression of experimentally induced pulmonary arteriosclerosis Circul Res N Y *3* 152 158 1955
8 Discussion by J F Dammann Jr and J E. Edwards of their papers Henry Ford Hospital International Symposium on Cardiovascular Surgery Philadelphia W B Saunders Co 1955 pp 117 118

EFFECT OF HEMIGASTRECTOMY, GASTRODUODENOSTOMY AND VAGOTOMY ON GROWTH IN PUPPIES*

JACK A. THOMSON, JOSEPH A. BONTA AND H. WILLIAM CLATWORTHY

Peptic ulcer during infancy and childhood is being recognized with increasing frequency. Since Cruveilhier[1] described ulcers occurring in infants age 1, 2 and 4 weeks nearly 130 years ago, several series of cases have been recorded.[2] While the great majority of these young ulcer patients can be carried through the critical years of growth on medical management alone, the catastrophic complications of this disease seen in both infancy and childhood occasionally leads to operative intervention. In these situations the problem of preventing recurrence while maintaining the growth potential becomes of immeasurable importance.

While the use of simple gastroenterostomy has almost always permitted adequate nutrition, the ultimate recurrence rate in these individuals is not known. The routine use of gastroenterostomy in young ulcer patients whose future life situation is unpredictable should ultimately lead to many operative failures. From the known high recurrence rate in adults after gastroenterostomy, it would seem likely that a significant number of the children would later require further operative intervention.

In theory this high recurrence rate could be overcome by partial gastric resection. Unfortunately, the nutritional problems seen in adults following the removal of 75 per cent or more of the stomach would be intolerable to the status of the growing child. The burden of rapid growth during childhood necessitates efficient digestion and absorption of food. This unique stress of growth would surely place a proportionately greater burden on the residual gastro-intestinal tract of a child. The undesirable nutritional results following gastric surgery would be expected to occur more frequently and be of a more critical nature. Most surgeons have accepted the probability of a high recurrence rate rather than submit the child to the possibility of a failure to gain weight. Gastroenterostomy rather than gastric resection is the usual operation performed in order to ascertain attainment of normal development.

The almost routine nutritional success and low ulcer recurrence rate noted in this medical center following hemigastrectomy, gastroduodenostomy and vagotomy in adults suggested that this operation might fulfill these requirements and constitute an acceptable operation for the child. A study of this procedure on the growing animal has been made.

METHOD

Healthy 12 to 20 kg. mongrel bitches in the later stages of gestation were selected from the animal farm. All were isolated from the main animal colony and given appropriate care to insure live birth and good neonatal care for the pups. Three weeks after birth 3 litters each containing 4 to 6 healthy puppies were selected. Each litter was divided into 2 groups as nearly equal in number and size as possible. All the pups in 1 group of each litter were submitted to 50 per cent gastric resection, gastroduodenostomy and vagotomy.

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Further corroborative information concerning the function of the pyloric antrum has been presented by Woodward and Tillmans Surgeons in general became acutely aware of the importance of the antral mucosa in the early 1910s as a result of the work of Allen and Welch The work of many since that time has confirmed the antrum to be a very effective endocrine organ mediating the gastric phase of hydrochloric acid secretion and has shown this phase to be inhibited by exposure of the antral mucosa to a strongly acid environment Data presented elsewhere during the present meeting confirms the importance of this concept in that Wangensteen and his group have utilized a wide resection of the acid secreting portion of the stomach retaining the pyloric antrum in continuity in a series of over 100 cases with no instance of recurrent ulcer

The concern of many surgeons over the satisfactory nutrition of patients after gastric resection for ulcer has led Thompson, Bonta, and Clatworthy to evaluate a less rigorous operation in terms of satisfactory growth of puppies Hemigastrectomy gastroduodenostomy and vagotomy as an operative procedure was found to permit normal growth

The work of Brackney and Thal strongly suggests that the duodenum plays essentially a similar role in gastric secretion to that played by the pyloric antrum

The fascinating studies of Randall and his associates on blood volume changes in the dumping syndrome are carried further by this group in the current series of papers by studies which indicate that under the circumstances of the dumping interval cardiac output and cardiac stroke volume are both markedly reduced It is again suggested that these changes may be responsible for the symptoms of the dumping syndrome

The surgical management of intestinal obstruction requires that the abdomen be closed with a fully emptied intestinal tract Lein and Maddock report successful removal of gaseous distention from colon and small intestine in dogs by multiple punctures with large bore needles without evidence of leakage or peritonitis thereafter Unfortunately the presence of much fluid interferes with successful decompression by this means

Cohn has added further confirmation of the observations of Poth and his associates of many years ago with regard to the role played by bacteria in strangulating small bowel obstruction

Perry has performed quantitative studies confirming the long appreciated importance of the last few centimeters of terminal ileum in the matter of water reabsorption

suggests that when great care is employed rather accurate estimates can be made In no instance was it necessary to remove more stomach

Table 1 Weight of Specimens in Grams—Per Cent of Stomach Removed by Weight

LITTER 7		LITTER 9
8 1 gms = 100%	TOTAL STOMACH	14 8 gms = 100%
4 0 gms = 49 %	DOG 1	6 6 gms = 44%
5 2 gms = 61%	DOG 2	8 1 gms = 55%

1 animal in each litter sacrificed

Table 2 Weight Gain in Kilograms

AGE AND WEIGHT

LITTERS	DOG	AGE IN WEEKS 2	4	8	20	28	36	40	48
6	1	.48		30		91	105	91	
	2	.57		23		73	86	7.3	
	3	50		28		86	98	84	
	4	49		29		84	91	7.7	
7	1	85	16	41	91	102	150		182
	2	.68	1.6	38	7.7	100	136		141
	3	76	17	36	77	103	136		150
	4	93	18	43	91	102	151		186
9	1		10	18	36	64			
	2		11	1.7	41	73			
	3		9	18	45	77			
	4		9	20	41	68			

EXPERIMENTAL ANIMALS

Table 2 is a summary of the weight of the animals at the various ages of life Initially each group of each litter is approximately equal in weight During a 4 to 8 week period after operation the gastrectomized animals lag behind the controls This probably is due to the loss of eating for several days and the adaptation by the animals to smaller and more frequent feedings Thereafter the animals with the stomach partially removed gained weight at a more rapid rate until at the end of the period of observation the 2 groups are approximately the same in weight In the final observation of litter 6 the 2 operated animals exceed the control pair by an average of 1 kg or 1 per cent Control animals of litter 7 exceed the operated animals by an average of 6 kg or 4 per cent while the control animals of litter 9 exceed the operated animals by an average of 1 kg or 5 per cent In overall averages the control exceeded the operative animals by less than 5 per cent

The smallest animal is only 15 per cent below the average weight of its controls and the largest operated animal is 9 per cent above the average weight of its controls In litter 6 the largest animal is an operated animal The range of variation between the 2 groups is quite small and indicates that nutrition was affected very little by the procedure The development in all the animals was comparable

Four hours prior to surgery the pups were isolated from the mother to insure an empty upper gastrointestinal tract. The puppies were then anesthetized with open drop ether, and anesthesia was maintained on a mixture of O_2 and ether delivered into a partially closed system. Depth of anesthesia was altered by changing the percentage of ether delivered. The abdomen was opened through a midline incision extending from the xiphoid to the umbilicus. The ensiform process was removed. The vagi were identified as they passed into the abdomen through the esophageal hiatus. Considerable difficulty was frequently encountered in locating the posterior vagus nerve. In puppies the diaphragm is quite thin and is easily perforated during the posterior dissection necessary to define the vagus.

Approximately 50 per cent of the stomach was removed by applying clamps in the midportion of the stomach. A single layer of 5-0 black silk was utilized to perform the anastomosis. The gastroduodenostomy was accomplished in the classic manner of Horsley[4] by closing the greater curvature side of the gastric pouch and anastomosing the duodenum to the lesser curvature of the gastric pouch. Abdominal closure was affected with through and through number 36 stainless steel wires. The operative procedure required an average of 35 to 45 minutes after induction of anesthesia.

Determination of the amount of stomach removed was initially based on the surgeon's estimate. It soon became apparent that a more quantitative method was needed. Maclean[5] has suggested that weight of the stomach removed in patients without gastric ulcer or duodenal obstruction is a reliable method to determine the amount of stomach removed. In dealing with a normal gastrointestinal tract it was felt that this method would be ideal. One animal of average weight and size was sacrificed in each of the later litters and the stomach weighed. At subsequent operations of litter mates specimens were removed that approximated 50 per cent of the weight of the control stomach.

Postoperatively the pups were returned to the mother when awake. Ordinarily spontaneous suckling began immediately. Those animals who had difficulty during the first days after operation were hand fed until they returned to health. Weening took place at age 6 to 8 weeks; thereafter all puppies were maintained on a similar laboratory diet consisting of cooked horsemeat and commercial dog food. All animals received passive immunization against distemper every 2 weeks until 3 months of age at which time active immunization was carried out. A vermifuge was administered every month after the pups attained the age of 2 months. At periodic intervals observations on weight and growth were made with no dietary supplements being given other than the immediate postoperative aid offered the operated animals. A comparison of growth in operated and control groups was made.

RESULTS

Table 1 presents a comparison of the weight of the total control stomach to the weight of the specimens removed in each of the litters. In each instance the surgeon applied the clamps so that an estimated 50 per cent of the weight of the stomach was removed. The smallest estimate made turned out to be 44 per cent by weight and the largest 61 per cent. This

3 Guthrie K Peptic ulcer in infancy and childhood with a review of the literature Arch Dis Childh Lond 17 82 1942
4 Horsley J Shelton Partial gastrectomy J Am M Ass 86 664 668 1926
5 Maclean L D and Lillehei R C A comparative evaluation of tubular gastric resection In Surgical Forum 1953 Philadelphia W B Saunders Co 1954 pp 285 291
6 Everson T C Experimental comparison of protein and fat assimilation after Billroth II Billroth I and segmental types of subtotal gastrectomy Surgery 36 2 1954

MECHANISM OF ULCER RECURRENCE FOLLOWING THE ANTRUM EXCLUSION GASTRECTOMY*

EDWARD R WOODWARD AND VICTOR C TILLMANNS

For more than half a century distal partial gastrectomy has been used in the surgical treatment of duodenal ulcer During this time surgeons have never ceased to search for a safe method by which the difficult posterior penetrating duodenal ulcer can be handled As a result of dissection around such a lesion leakage from a duodenal stump and reactive pancreatitis have contributed in large measure to the morbidity and mortality of patients who undergo partial gastrectomy For a time gastroenterostomy was thought to be the solution to this problem The marginal ulcer rate however proved to be prohibitively high when this procedure was used for active duodenal ulcer Since Dragstedt s[1] introduction of vagotomy in 1943 this procedure combined with gastroenterostomy or pyloroplasty has been accepted by many surgeons as the procedure of choice in dealing with a duodenal ulcer of this difficult nature

Von Eiselsberg proposed the antrum exclusion operation in treatment of the posterior penetrating duodenal ulcer He transected the stomach at approximately the level of the incisura, closed the distal end and performed end to-side gastrojejunostomy with the proximal end of the stomach as in the Polya modification of the Billroth II operation This procedure avoided the difficult dissection in the region of duodenal ulceration Since the antrum was known to secrete no hydrochloric acid von Eiselsberg planned to divert the acid gastric juice away from the duodenum and thus allow prompt healing of the ulcer

In 1918 Finsterer[3] modified von Eiselsberg s operation in what he called his Resection for Exclusion In a patient whose duodenal ulcer precluded safe dissection at the pylorus, the stomach was *divided several* centimeters proximal to the pylorus leaving a distal cuff of antrum *uninvolved* in the ulcerative process which could be closed easily and safely A sleeve area of the stomach was then excised thus removing a portion of *the* acid secreting mucous membrane Gastrojejunostomy was performed after the method of Polya As late as 1931 *Finsterer*[4] was *still* advocating his Resection for Exclusion without excision of the antrum mucosa

From the Department of Surgery University of California Medical Center at Los Angeles and the Wadsworth General Hospital Veterans Administration Center Los Angeles California This work has been supported by Grant Number G 4195 United States Public Health Service

DISCUSSION

The complications of peptic ulceration in childhood presents a complex problem Nutrition and growth potential must be maintained despite the ultimate probability of a high rate of recurrent ulceration With the relatively high number of nutritional failures in adults following a 75 per cent or greater gastric resection most surgeons have resorted to gastroenterostomy even in the presence of medically uncontrolled hemorrhage though it is well recognized that removal of the bleeding area offers the best chance for survival Nutritional success and low recurrence rate attained in our clinic while managing adults suggested that hemigastrectomy vagotomy and gastroduodenostomy might allow normal growth

The results indicate that this combination of hemigastrectomy vagotomy and gastroduodenostomy allows the maintenance of growth potential and nutrition in a period when growth is maximal The fact that some of the operated animals exceed the controls in weight and the average variation of weight between the control and operated groups is less than 5 per cent indicates that this operation interferes very little with the future nutritional status of the animals The ability of the animals to continue normal development is of noteworthy importance

While no determinations were carried out in this group the work of Iverson[6] suggests that the probable reason for maintenance of normal nutrition is a relatively good absorption of nitrogen and a relatively small loss of fat in the feces after hemigastrectomy and gastroduodenostomy The absorption of nitrogenous or protein containing material would appear to be of increased importance during growth The nutritional success attained in growing animals with limited resection suggest that such an operation should be considered for use in the child After further study in animals and better follow up in children subjected to gastroenterostomy or gastroenterostomy and vagotomy in the past such an operation may be more widely employed in treating complications of acid peptic disease in childhood

The results further suggest that the combination of hemigastrectomy, vagotomy and gastroduodenostomy have permitted adequate nutrition even under the unique physiological stress of growth The results are another indication of the physiological soundness of limited resection in combination with restoration of normal intestinal continuity

SUMMARY

1 Hemigastrectomy vagotomy and gastroduodenostomy in growing animals did not alter development

2 Weight variation between the experimental and control groups was less than 5 per cent at maturity

3 As growth is one of the severest physiological stresses the use of growing animals to evaluate nutritional efficacy of operative procedures is suggested

REFERENCES

1 Cruveilhier J Anatomie Pathologique du Corps Humain Paris J B Balliere 1829 42 Vol 2 pt 6

2 Bird C E Limper M A and Mayer J M Surgery in peptic ulceration of stomach and duodenum in infants and children Ann Surg *114* 526 1941

Since the antrum exclusion operation changed the antrum from an acid into a neutral alkaline environment, the present study was undertaken to investigate the possibility that lack of acid inhibition accounted for the unsatisfactory clinical results.

METHOD

Four dogs were prepared with a denervated accessory stomach (Heidenhain) pouch. Changes in secretion of acid gastric juice by this pouch were used as a measure of hormonal activity in the antrum. The intestinal phase of secretion was considered to be constant throughout the study. Either at the same operation or as a second stage procedure the stomach was divided in its mid portion, the distal end closed and the proximal stomach anastomosed end-to-side with the first loop of jejunum (Fig. 1a). This is the operative procedure advocated by von Eiselsberg and Devine and it preserves a cuff of acid secreting gastric mucosa along with the excluded antrum.

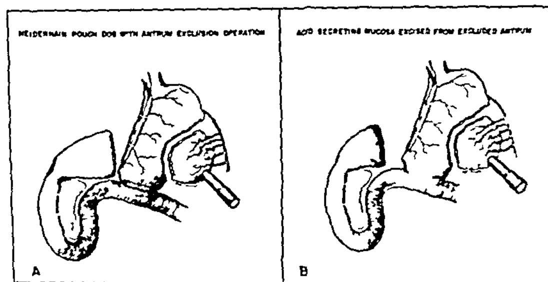


Fig. 1. Diagrams of surgical procedures performed showing (a) the antrum excluded along with a cuff of acid secreting body and (b) the acid secreting portion removed from the excluded antrum.

Quantitative 24 hour collections of gastric juice were made from the Heidenhain pouch after the method of Dragstedt, Haymond and Ellis.[11] The animals were maintained on a constant dietary intake with electrolyte balance preserved by addition of sodium chloride to the ingested food in amounts equivalent to the chloride lost.

After a control period of 19 to 57 days each animal was reoperated upon. The cuff of acid secreting mucous membrane attached to the excluded antrum was resected and the proximal end of the excluded antrum closed (Fig. 1b). Microscopic sections confirmed the removal of all acid secreting mucosa from the antrum in each case. Following recovery from this procedure the animals were maintained on the previous dietary intake during a control period of similar length.

RESULTS

In all 4 animals removal of the acid cuff from the excluded antrum was followed by a moderate increase in secretion of acid gastric juice from the

In 1925 Devine[5] reported his Alkalizing Operation He pointed out that in the old callous ulcer which was situated on the posterior duodenal wall recurrence frequently followed gastroenterostomy and resection was difficult and dangerous During the preceding 9 years he had divided the stomach in its mid portion in 30 cases, closed the distal end and performed a Polya gastrojejunostomy without resecting any stomach In essence this is the procedure advocated by von Eiselsberg Devine felt that by means of this technique adequate alkaline regurgitation into the proximal stomach could be ensured thus neutralizing hydrochloric acid output and the ulcer could be closed off in a dead end In 3 of the 30 patients there was recur *rence of melena during the postoperative period The acidity of the stomach* was found to be reduced as much as or more than in the usual type of Polya gastric resection

Dissatisfaction with the antrum exclusion operation was a long time in appearing In 1936 Ogilvie[6] reported his Physiological Gastrectomy which was nearly identical with the Resection for Exclusion of Finsterer He felt that preservation of the antrum might prevent some of the untoward nutritive effects of radical gastrectomy Two years later Ogilvie[7] retracted his recommendation for this procedure and stated that with wider knowl edge he now realized that exclusion operations should at all cost be avoided Of 22 patients having this operation 9 developed marginal ulcers He noted that gastrin had been found only when food came in contact with the pyloric mucous membrane and therefore gastrin should not be produced by a pyloric segment permanently excluded from the food channel A study of patients after exclusion gastrectomy, however showed a high acid curve in marked contrast to the low figure seen after the usual type of gastric resection He postulated that the secretory hormone may be manu factured in the pylorus in response to stimuli other than food such as muscular contractions or the regurgitation of bile from the duodenum

In 1942 Allen and Welch[8] reported a large series of subtotal gastric resections performed for duodenal ulcer The poorest results were sustained by those patients who had resection for exclusion without removal of the antral mucosa Nine such operations were performed and 5 of the 9 patients developed jejunal ulcer In the same year Wangensteen[9] reported on 15 patients who had the antrum exclusion operation in which the antral mucosa was coned out before closing the excluded antrum Bancroft is generally credited with originating this technique Unlike patients whose antral mucosa was retained postoperative gastric acids were low and none of these patients developed marginal ulcer

Since Edkin s original statement of the gastrin hypothesis in 1906[10] *efforts* to substantiate his work have been unsuccessful Motivated partly by the suggestive clinical facts noted re investigation of this problem was under taken in the laboratory in 1947 In a series of experiments on dogs it has been established that the antrum functions as an endocrine organ producing a hormonal substance the gastrin of Edkins which is a powerful stimulant of gastric secretion [11] Further experiments have suggested that the presence of acid inhibits the hormonal function of the antrum [12] More recently we have demonstrated that application of acid food material to the isolated antrum fails to elicit the secretion of acid gastric juice by the main stomach [13] We have theorized that this constitutes a normal physiological mechanism terminating the gastric phase of gastric secretion

resected the mid portion of the stomach leaving only the non acid secreting antrum or a portion thereof as a blind end Devine on the other hand resected no stomach and left a substantial amount of acid secreting mucous membrane attached to the excluded distal portion of the stomach

In the experiments herein reported Heidenhain pouches were constructed in dogs for use as an indicator of variations in the antral hormonal mechanism The stomach was divided in its mid portion excluding a segment of acid secreting gastric mucosa along with the antrum of the stomach This is essentially the procedure advocated first by von Eiselsberg and later by Devine At a second procedure the acid secreting mucosa was excised from the excluded antrum This left the excluded antrum in a neutral or alkaline environment as in resection for exclusion as advocated by Finsterer Following excision of the acid secreting mucous membrane from the excluded antrum there was a 36 to 56 per cent increase in secretion of acid gastric juice by the Heidenhain pouch

The results support the hypothesis that pH is an important regulating device for the endocrine function of the pyloric antrum When the antrum is excluded in the absence of acid secreting mucosa a neutral or alkaline environment prevails Food substances are known to reflux for considerable distances in the gastrointestinal tract and apparently will reflux through the afferent jejunal loop and duodenum into the excluded antrum Therefore the chemical stimulation of foodstuffs is present in the antrum and the normal shut off device of a drastically reduced pH is absent This allows hyper function of the antral hormonal mechanism releasing gastrin and resulting in prolonged and excessive stimulation of secretion of acid gastric juice

On theoretical grounds the operation of von Eiselsberg and Devine where acid secreting mucosa is included with the antrum is preferable to the Finsterer resection However in the duodenal ulcer patient where posterior penetration makes resection undesirable the excessive interdigestive secretion of acid gastric juice can be drastically reduced by vagotomy This procedure combined with a drainage operation is considered preferable to any type of exclusion procedure in the surgical treatment of such a duodenal ulcer

SUMMARY AND CONCLUSIONS

The incidence of recurrent peptic ulceration following the antrum exclusion type of gastric resection is known to be high The hormonal mechanism by which the antrum stimulates secretion of acid gastric juice is inhibited at a low pH It is postulated that in the excluded antrum absence of acid gastric juice causes prevalence of a neutral or alkaline environment Food substances refluxing through the afferent loop stimulate the antral mechanism and failure of normal shut-off follows because of the absence of HCl

REFERENCES

1 Dragstedt L R and Owens F M Supra-diaphragmatic section of the vagus nerves in treatment of duodenal ulcer Proc Soc Exp Biol N Y *53* 152 154 1943

2 von Eiselsberg A Zur unilateralen Pylorusausschaltung Wien Med Wschr *23* 44-48 1910

3 Finsterer H Ausgedehnte Magenresektion bei Ulcus duodeni statt der einfachen Duodenalresektion bzw Pylorusausschaltung Zbl Chir *45* 434-435 1918

Table 1 The Effect of Excising Acid Secreting Mucosa from the Excluded Antrum

ANIMAL	NO OF COLLECTIONS	AVERAGE VOL (cc)	AVERAGE FREE ACID (mEq/l)	AVERAGE 24 HOUR HCl OUTPUT (mEq)
BEFORE				
1	50	359	125	46.2
2	57	129	110	14.7
3	49	159	120	20.2
4	50	108	90	9.7
AFTER				
1	59	490	138	68.8
2	18	160	121	20.0
3	53	211	133	32.7
4	55	151	104	16.1

Heidenhain pouch (Table 1) The increase in average daily output of HCl is demonstrated graphically in Figure 2 The output of acid gastric juice by the Heidenhain pouch was increased by 36 per cent 19 per cent 62 per cent and 66 per cent respectively In 2 of the 4 animals bleeding occurred intermittently from the pouch after resection of the acid cuff attached to the excluded antrum This indicated that pouch secretion had become elevated to ulcerogenic levels The complication of peptic ulcer within the pouch limited the observation period to 18 days in 1 animal (Number 2)

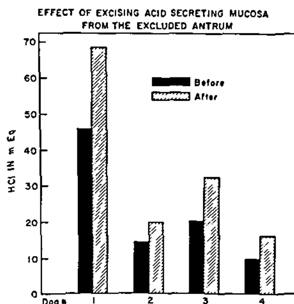


Fig 2 Effect of excising acid secreting mucosa from the excluded antrum

DISCUSSION

In recent years it has been customary to refer to any gastric resection preserving an antral segment as a Finsterer Devine exclusion operation There is however a marked technical difference in the procedure advocated by these two men which has important theoretical implications Finsterer

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1 Dragstedt L R and Owens F M Supra diaphragmatic section of the vagus nerves in treatment of duodenal ulcer Proc Soc Exp Biol N Y *53* 152 154 1943

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3	53	241	133	32.7
4	55	151	104	16.1

Heidenhain pouch (Table 1). The increase in average daily output of HCl is demonstrated graphically in Figure 2. The output of acid gastric juice by the Heidenhain pouch was increased by 36 per cent, 19 per cent, 62 per cent and 66 per cent respectively. In 2 of the 4 animals bleeding occurred intermittently from the pouch after resection of the acid cuff attached to the excluded antrum. This indicated that pouch secretion had become elevated to ulcerogenic levels. The complication of peptic ulcer within the pouch limited the observation period to 18 days in 1 animal (Number 2).

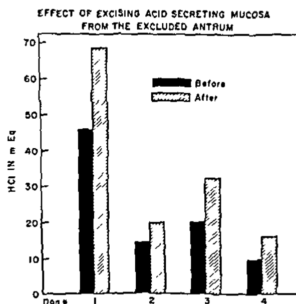


Fig. 2. Effect of excising acid secreting mucosa from the excluded antrum.

DISCUSSION

In recent years it has been customary to refer to any gastric resection preserving an antral segment as a Finsterer-Devine exclusion operation. There is, however, a marked technical difference in the procedure advocated by these two men which has important theoretical implications. Finsterer

Table 1. Occurrence of Curling's Ulcer
(January 1951 - September 1955)

PATIENT	AGE	SEX	PERCENTAGE: BODY SURFACE BURNED	PERCENTAGE: THIRD DEGREE BURN	BLEEDING: POSTBURN DAY	DEATH: CAUSE	DEATH: POST BURN DAYS	SITE OF ULCER	REMARKS
1 G S	37	M	23	17	16th	survived		unknown	3,000 cc blood, open fract. F. L.
2 J L	25	M	30	20	none	septicemia	102	duodenum & stomach	perforation and abscess at autopsy
3 P B	22	M	35	15	12th	survived		unknown	3,000 cc blood in 12 hours
4 W M	36	M	58	58	7th	hemorrhage*	7	unknown	no autopsy
5 F D	55	F	60	40	none	septicemia	20	duodenum	cortisone therapy asymptomatic
6 H V	21	M	72	43	23rd	survived*		unknown	massive hemorrhage* 3,000 cc blood
7 F S	27	M	72	20	19th	peritonitis	30	duodenum	gastrectomy, poor healing
8 M K	37	F	82	64	5th	pulmonary edema	9	duodenum & stomach	
9 E C	34	M	90	55	2nd	undetermined	3	entire bulb of duodenum	
10 L N	36	M	95	90	none	septicemia	9	early ulceration duodenum	erosion of esophagus
Average			61.2	42.2					

* Septicemia present

4 Finsterer H and Cunha F The surgical treatment of duodenal ulcer Surg Gyn Obst 52 1099 1114 1931
5 Devine H B Basic principles and supreme difficulties in gastric surgery Surg Gyn Obst 40 1 16 1925
6 Ogilvie W H Physiology and the surgeon Edinburgh M J 43 61 83 1936 Series 3
7 — The approach to gastric surgery Lancet Lond 2 295 299 1938
8 Allen A W and Welch C E Gastric resection for duodenal ulcer follow up studies Ann Surg 115 530 543 1942
9 Wangensteen O W Method of closing the pyloro antral pouch in the antral exclusion operation Surgery 12 731 741 1942
10 Edkins J S The chemical mechanism of gastric secretion J Physiol Lond 34 133 144 1906
11 Woodward E R Bigelow R R and Dragstedt L R Effect of resection of antrum of stomach on gastric secretion in Pavlow pouch dogs Am J Physiol 162 99 109 1950
12 Oberhelman Jr H A Woodward E R Zubiran J M and Dragstedt L R Physiology of the gastric antrum Am J Physiol 169 738 748 1952
13 Woodward E R Lyon E S Landor J and Dragstedt L R The physiology of the gastric antrum Gastroenterology 27 766 785 1954
14 Dragstedt L R Haymond H E Ellis J C Cannula gastrostomy and enterostomy Surg Gyn Obst 56 799 801 1933

STUDIES IN CURLING S ULCERS*

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During the 21 month period from January 1954 to September 1955 10 cases of gastrointestinal ulceration occurring after burns have been seen among 194 hospitalized burned patients at Brooke Army Hospital (5 1 per cent) Of the 31 deaths following burns occurring during the same period 7 patients were found to have gastrointestinal ulcerations (22 6 per cent) This incidence is similar to that previously reported from this hospital by Weigel [1] covering a 3 year period from 1950 to 1953 in which 7 ulcerations were seen following 27 deaths

In only 2 instances (Cases 1 and 7 Table 1) were the ulcerations thought to be the immediate cause of death The patients who developed Curling s ulcers had extensive burns — average total of body surface burn was 61 per cent including a third degree average of 42 per cent Patients 1 2 and 3 had smaller burns but each had an added insult Patient 1 had an open fracture of the 12th thoracic vertebra and patients 2 and 3 had septicemia Three of the patients survived The development of ulcers in the more severely burned patient generally occurred during the first and second post burn week In the less severely burned evidence points towards the development of the ulcer after the second or third week

Uropepsin Studies Theories as to the genesis of Curling s ulcers are numerous They were reviewed by Harkins in 1938 and Friesen in 1950 [3] The concept has developed in recent years that acute gastrointestinal ulceration following burns is a type of stress ulcer associated with increased production of adrenocortical hormone [4] Gray and others[5 6 7] have noted that uropepsin excretion gave an accurate estimate of the peptic activity of the stomach and that a high content of this enzyme accompanied

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Patient	Age	Sex	Percentage: Body surface burned	Percentage: Third degree burn	Post burn day	Volume of urine ml./24 hrs	Uropepsin mg./24 hrs	Gastric content: Acidity Free	Gastric content: Acidity Total	Gastric content: Benzidine test	Eos count	Expired	Remarks
						Highest uropepsin levels							
15 W H	18	M	40	17	9th	6 020	1 700	18	42*		0	no	
16 O W	23	M	40	25	29th	2 9[illegible]0	1 904	7	26	pos	66	yes	septicemia severe
17 H W	21	M	40	25	60th	2 910	1 113	0*	29		22	no	after cortisone therapy
18 O U	21	M	40	40	68th	3 680	1 162	0	5		300	no	arrived late infect
19 W J	18	M	45	5	28th	2 880	1 162	39	87	tr		no	after discontinuing banthine
20 W I	23	M	45	15	22nd	3 580	2 855	0	3		200	no	
21 C M	24	M	50	20	25th	1 100	3 706	0	13		0	yes	septicemia severe
GROUP C													
22 M C	21	M	55	45	15th	3 360	582	0	17	pos	[illegible]0	yes	septicemia a [illegible] on 11th postburn day
23 I D	55	F	60	40	19th	1 980	526	32	56			yes	Curling's ulcer septicemia cortisone for 1st 8 days
24 M K	37	F	82	64	5th	915	1,29[illegible]	38	63	pos	0	yes	Curling's ulcer
25 M R	26	F	90	50	2nd	266[illegible]	526	36	67*		[illegible]	yes	septicemia
26 F C	34	M	90	55	3rd	1 190	[illegible]	22	[illegible]	pos	33	yes	Curling's ulcer
27 I N	36	M	95	91	2nd	[illegible]10	552	19	9	pos	22	yes	Curling's ulcer septicemia

Table 2 Studies in Uropepsin Levels

				PERCENTAGE		HIGHEST UROPEPSIN LEVELS									
									GASTRIC CONTENT						
									ACIDITY						
	PATIENT	AGE	SEX	BODY SURFACE BURNED	THIRD DEGREE BURN	POST BURN DAY	VOLUME OF URINE ML/24 HRS	URO PEPSIN MG/24 HRS	FREE	TOTAL	BENZIDINE TEST	EOS COUNT	EXPIRED	REMARKS
	GROUP A													
1	J I	23	M	5	3	11th	2,500	364	0	9	pos	200	no	
2	A M	25	M	9	3	4th	1 760	465	12	36		12	no	
3	R I	24	M	10	0	2nd	2 620	443				50	no	
4	T S	30	M	15	0	5th	3 450	873	0	16	pos	38	no	deep 2 burn
5	D H	26	M	20	8	2nd	2 670	838				0	no	deep 2° burn in 3 areas
6	C H	22	M	20	8	35th	3 400	1,304	29	51°	pos	165	no	transient septicemia
7	B I	26	F	21	5	8th	3 735	760	0	2		33	no	
8	J N	21	M	21	10	14th	3 200	866				279	no	deep burn in 3 areas white phosphorus
9	M K	20	M	22	7	34th	2 480	504	0	10	tr	99	no	bacteremia
10	D M	27	M	22	10	28th	4 570	1 026	0	17	pos	250	no	deep burn in 3° areas white phosphorus
11	K H	22	M	25	0	5th	3 020	824	6	8°		25	no	deep 2° burn
12	V C	23	M	25	10	17th	3 730	741				187	no	
	GROUP B													
13	H A	77	F	30	10	3rd	975	77				0	yes	coronary occlusion
14	H C	23	M	35	20	20th	1 740	1 100	30	47°	pos	175	no	..

definite correlation between uropepsin excretion and the development of the ulcer However all 4 patients had overwhelming burns Each of these patients had elevated gastric acidity but some extensively burned patients who did not develop gastrointestinal ulcerations also showed elevated gastric acidity

Eosinophil counts in general remained low during the first postburn week and began to return to normal during the second and third postburn weeks unless severe infection was present There was no consistent relationship between eosinophil counts and uropepsin excretion

Patients 15 20 and 22 had simultaneous uropepsin and urinary steroid determinations performed The 17 ketosteroids were not remarkably elevated However the 17 hydroxycorticoids and 11-oxycorticoids were elevated when the uropepsin levels were elevated and when the uropepsin began to fall there was a proportionate lowering of the levels In patient 22 both the corticoids and the uropepsin fell terminally as the kidney function became markedly impaired

Cortisone was given to patient 17 who had a proven adrenal insufficiency and it immediately caused an elevated excretion of uropepsin Banthine was given to patient 19 because a moderate amount of blood was seen in his Levine tube A marked drop in the amount of gastric secretion and gastric acidity followed the administration of Banthine At this time the uropepsin was low therefore the Banthine did not seem to affect it After the Banthine was discontinued the gastric acidity gastric pepsin and uropepsin increased appreciably

DISCUSSION

It is difficult to evaluate the results of the uropepsin excretion in burns Several factors influence the uropepsin output other than the extent of burn The depth of the burn amount of infection age of the patient medication associated injuries and even emotional factors seem to influence uropepsin excretion [10]

In this preliminary study uropepsin excretion and gastric acidity determinations gave no indications as to which burned patient might develop Curling s ulcer

From our experience it appears that the incidence of Curling s ulcer in burns is increasing This may be attributed to advances in supportive therapy which have increased the survival time of the severely burned patient and thus he becomes a possible candidate for the development of an ulcer

Since Curling s ulcer is one of the dangerous complications of extensively burned patients its possible development should be considered Early signs however are difficult to determine Usually there is little indication of the development of an ulcer until a massive hemorrhage occurs When treating extensive burns it might be wise to give antacids prophylactically during the first 2 or 3 weeks

SUMMARY

1 Ten cases of gastrointestinal ulceration occurred among 191 burned patients seen over a 21 months period (5 1 per cent) Of 31 deaths following burns 7 patients were found to have Curling s ulcers

2 In a series of 27 hospitalized burned patients the uropepsin excretion

peptic ulcer They also noted that uropepsin excretion was increased by adrenal and pituitary hyperactivity stress response, and ACTH and corti sone therapy Gray[8] mentioned one patient with an undisclosed amount of second degree burn who showed an elevated uropepsin level at the end of the first postburn week

The purpose of this study was to correlate the peptic activity in the stomach (as measured by pepsinogen levels in the urine) the level of gastric acidity and eosinophil count with the extent of burn and the development of Curling s ulcer in a group of hospitalized burned patients

Twenty seven moderately to severely burned patients were studied Uropepsin levels were determined by the method of Bucher[9] utilizing a hemoglobin substrate It was assumed after the findings of Bucher that when renal function was normal the enzyme was eliminated by the kidney without difficulty and that the normal values for uropepsin levels ranged from 35 to 876 mg of tyrosine per 24 hours Basal and nocturnal gastric secretions were analyzed for free and total acidity and for the presence of blood Normal free acid was considered to range from 0 to 30° and total acid from 5° to 40°

RESULTS

Table 2 lists the highest uropepsin output for each patient studied, and the volume of urine gastric acidity benzidine reaction and eosinophil count for the same day The patients were grouped into 3 categories according to the size of total burn Group A included burns involving less than 30 per cent of the body surface Group B included burns involving 30 to 50 per cent of the body surface and Group C burns covering more than 50 per cent of the body surface In the less severe burns (Group A) uropepsin excretion was at a normal or high normal level unless some added systemic insult such as septicemia (patient 6) was present In the moderately severe burns (Group B), uropepsin excretion was considerably elevated except in a 77 year old patient who had a 30 per cent burn (patient 13) In the more extensive burns (Group C) uropepsin excretion was not elevated This may be the result of diminished renal function in an extensively burned patient rather than failure of the gastric glands to secrete pepsin This finding requires further investigation and measurement of pepsinogen levels in gastric secretion

Patients with second degree burns seemed to reach a peak of uropepsin output during the first and second postburn weeks These levels usually returned to normal by the end of the third week at the time when the wounds were healed Patients with third degree burns tended to have a greater uropepsin output during their second third and fourth postburn weeks at which time the third degree wound was sloughing and considerable infection was present These patients tended to run elevated uropepsin levels far into the grafting period even if this phase lasted as long as 3 months

There was considerable variability in gastric acidity although there was a trend toward higher gastric acidities in the more severely burned patients

All the patients who had a benzidine test performed on their gastric secretion were found to have a trace to a strongly positive reaction Four patients in this study developed Curling s ulcer There seemed to be no

secretory response following the induction of insulin hypoglycemia. The recent observations of Shay and Sun[2] upon a group of patients with histories of chronic duodenal ulcers supported these findings.

In the present experimental study we have investigated the possibility of direct hypothalamic control of gastric secretory stimulation mediated by the pituitary stalk or accompanying hypophyseal portal vessels. The pituitary stalk was sectioned by the transtemporal route and a tantalum plate inserted as a barrier to regeneration of the hypophyseal portal system in 12 dogs (8 with simple gastric fistula and 4 with total gastric pouch). Harris and Jacobsohn[3] demonstrated the regeneration of direct vascular connections between the hypothalamus and the pituitary gland after complete section of the pituitary stalk. They were able to prevent such regeneration by the use of a barrier.

Subsequent to section of the pituitary stalk in dogs with simple gastric fistula, collections of gastric juice revealed statistically significant variations in gastric secretory volume as well as sodium and potassium concentration as compared to values obtained prior to the stalk section. Six dogs prepared with simple gastric fistula were selected for these studies on the basis of radiological evidence of satisfactory positioning of the tantalum plate barrier. Five or more secretion periods were studied in these animals. Following the pituitary stalk section, mean gastric secretory volume was observed to decrease by 39 per cent, mean sodium concentration decreased by 26 per cent while a reduction of 32 per cent was noted in mean potassium concentration. The gastric acidity was increased. Mean free acid concentra-



Fig. 1. Operative exposure of pituitary stalk by a transtemporal approach. The procedure employed has been described in detail by Rothballer and Skoryna. (a) Dural opening. (b) Pituitary gland. (c) Pituitary stalk. (d) Third nerve. (e) Carotid artery.

was found to be elevated in patients with burns involving from 30 to 50 per cent of the body surface However, in burns covering more than 50 per cent of the body surface the uropepsin levels were not elevated It was the clinical impression that this lack of elevation may be due to renal impairment Gastric acidity varied greatly in these patients although there was a trend toward higher gastric acidity in the more severely burned patients There seemed to be some correlation between urinary pepsin and steroid excretion but less with eosinophil level

3 It was not possible to predict from the uropepsin and gastric acidity levels which patient would develop Curling s ulcer

REFERENCES

1 Weigel A E Artz C P Reiss E Davis J H and Amspacher W H Gastrointestinal ulcerations complicating burns Surgery *34* 826 36 1953
2 Harkins H N *Acute ulcer of the duodenum (Curling s ulcer)* as a complication of burns relation to sepsis Surgery *3* 608-41 1938
3 Friesen S R The genesis of gastroduodenal ulcer following burns Surgery *28* 123 58 1950
4 Fletcher D C and Harkins H N Acute peptic ulcer as a complication of major surgery stress or trauma Surgery *36* 212 25 1954
5 Gray S J Benson J A Jr Reifenstein R W and Spiro H M Chronic stress and peptic ulcer J Am M Ass *147* 1529 37 1951
6 Janowitz H D and Hollander F Relation of uropepsinogen excretion to gastric pepsin secretion in man J Appl Physiol *4* 53 6 1951
7 Podore C J Broh Kahn R H and Mirsky I A Uropepsin excretion by man III Uropepsin excreted by patients with peptic ulcer and other lesions of the stomach J Clin Invest *27* 834 839 1948
8 Gray *S J* Ramsey C G and *Reifenstein* R W *Clinical use of the urinary uro*pepsin determination in medicine and surgery N England J M *251* 835-43 1954
9 Bucher G R Uropepsin a review of the literature and report of some experimental findings Gastroenterology *8* 627-47 1947
10 Gray S J *et al* The significance of hormonal factors in the pathogenesis of peptic ulcer Gastroenterology *25* 156 1953

GASTRIC SECRETION STUDIES FOLLOWING PITUITARY STALK SECTION IN DOGS*

THOMAS A JOHNSON JR STANLEY C SKORYNA ALAN B ROTHBALLER AND DONALD R WEBSTER

Hypothalamic centers may bring about changes in gastric secretory activity by transmission of autonomic impulses over vagal and sympathetic routes or by a hormonal route via the adenohypophyseal adrenocortical axis As knowledge developed concerning the pituitary adrenocortical activation of gastric secretion in response to various forms of stress it appeared that the relationship between these secretory changes and conditions favoring development or chronicity of peptic ulcer should be of interest In experimental studies utilizing anesthetized monkeys French Longmire Porter and Movius[1] found that intact adrenal cortex is essential for the normal gastric

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Fig 2 X ray view of the skull of Dog #51 The position of the tantalum plate barrier is shown following the section of the pituitary stalk

One hour collections of gastric juice were obtained before and for a period of 5 hours after the induction of insulin hypoglycemia in a group of 8 animals On repeating these studies following section of the pituitary stalk it was necessary to decrease the dosage of insulin from an average of 30 units to 10 units in order to attain comparable blood sugar levels at 1 or 2 hours following the administration of insulin The mean values of gastric secretory volume free acid total acid pH sodium potassium chloride and pepsin were compared separately with the equivalent values subsequent to pituitary stalk section In none of the variations in gastric secretory components during the first 5 hours following the induction of hypoglycemia could a statistically significant change be shown as following the stalk section

Satisfactory series of 24 hour collections of gastric juice before and after pituitary stalk section were obtained in 3 of the total gastric pouch animals with intact vagal innervation In 1 animal (Dog Number 51) the mean volume of gastric juice decreased from 175 to 106 ml per 24 hours An increase in gastric free acidity from a mean of 80 to 91 clinical units was noted The secretory pattern of this animal resembled that found in the simple gastric fistula animals (Table 1) A second animal (Dog Number 13) showed more marked decrease in mean secretory volume from 537 to 238 ml per 24 hours The concentration of free acid was unchanged by stalk section although pepsin concentration decreased by one third In this animal the interpretation of these changes is somewhat less certain in view of transient cerebral edema during the first week of the postoperative period The third total gastric pouch animal (Dog Number 48) was found to possess histologically intact pituitary portal vessels This secretory pattern differs markedly from the other animals studied The mean gastric secretory volume increased from 193 to 312 ml per 24 hours following the sectioning

tion was found to be 67 per cent higher and mean total acid concentration 28 per cent higher The mean free acid output was increased by 51 per cent The concentrations of gastric chloride and pepsin were found to be only slightly affected

The daily intramuscular administration of pitressin to 4 of the animals with pituitary stalk section resulted in restoration of the gastric secretory volume towards normal Statistically significant variations occurred in secretory volume free acid concentration total acid concentration and chloride concentration The mean gastric volume increased by 132 per cent Mean free acid concentration decreased by 68 per cent while mean total acid concentration decreased by 51 per cent The concentrations of sodium potassium and chloride were reduced by 13 30 and 36 per cent respectively The pepsin concentration increased by 91 per cent

Table 1 Effect of pituitary stalk section on gastric secretion in dogs with a gastric fistula Secretory volume is expressed in ml, acidity as clinical units per 100 ml of gastric juice electrolytes in mEq /l, and pepsin in mg of pepsin nitrogen per ml

	BEFORE	AFTER	CHANGE	t	P
Volume	29.7	18.1	—39%	3.416	.02
Free Acid	30	50	67%	1.967	.20
Total Acid	64	82	28%	1.567	.20
Gastric pH	2.53	2.27		0.409	.70
Sodium	55.08	40.97	—26%	3.093	.05
Potassium	12.71	8.61	—32%	3.473	.02
Chloride	122.5	133.9	9%	2.236	.10
Pepsin	0.981	0.919	—3%	0.092	1.00

Table 2 Effect of pitressin administration on gastric secretion in pituitary stalk sectioned dogs with a gastric fistula Secretory volume is expressed in ml acidity as clinical units per 100 ml of gastric juice electrolytes in mEq /l and pepsin in mg of pepsin nitrogen per ml

	BEFORE	AFTER	CHANGE	t	P
Volume	13.3	30.8	132%	2.857	.07
Free Acid	38	12	—68%	6.360	.01
Total Acid	71	35	—51%	3.901	.05
Gastric pH	2.78	3.90		1.868	.20
Sodium	44.42	38.43	—13%	1.229	.40
Potassium	8.20	5.72	—30%	2.221	.20
Chloride	128.5	82.7	—36%	4.026	.05
Pepsin	0.746	1.422	91%	1.060	1.00

importance of histamine in the etiology of experimental peptic ulcer is well known[6] Among the metabolites responsible for the development of gastro intestinal lesions during the alarm reaction special attention has been called to histamine which is liberated in excess or activated in various forms of tissue injury The exact place of histamine in the general endocrine response to stress is not well understood[4] Is histamine only an end product of tissue metabolism altered by pituitary adrenal stimulation or an important stressor stimulating the pituitary gland?

Some studies indicate that exogenous histamine results in a discharge of ACTH even in the absence of neural connections between the hypothalamus and the anterior pituitary[1] The stimulating effect of histamine on anterior pituitary transplants[2] and on the isolated perfused adrenals[4] was also reported Some reports seem to indicate that the reaction of animal organism to histamine depends upon the integrity of adrenal gland[10] The role of histamine in the physiological exocrine and endocrine activity of gastric mucosa is however unknown and the relationship of histamine to endocrine glands is not satisfactorily explained

The present paper records the results of stimulation of gastric endocrine function by ACTH and histamine in normal hypophysectomized adrenalectomized and thyroidectomized dogs Gastric endocrine function was studied by determination of plasma pepsinogen

METHOD

Twenty adult mongrel dogs of both sexes fed on commercial dog food and weighing from 6 to 20 kg were used in this experiment They were operated under sodium pentobarbital anesthesia (35 mg per kg of body weight) after a fasting period of 24 hours

Hypophysectomy was accomplished in 5 dogs through a right subparietal craniectomy No hormonal therapy was given after the operation and the present experiment was carried out from 5 to 6 months after hypophysectomy The dogs were sacrificed 4 weeks after the experiment There was no trace of pituitary tissue in dogs 8 9 and 10 Minute residual foci of glandular cells were found in dogs 6 and 7 As all dogs of this group were comparable in their response to the stimulants used the physiological significance of pituitary remnants noted in 2 dogs is presumably negligible

Thyroidectomy was performed in 5 dogs by a procedure which preserved at least 2 parathyroids The dogs were used in the present study from 8 to 11 months after thyroidectomy

Bilateral adrenalectomy was carried out as a one stage procedure preceded by the administration of cortisone 25 mg daily for 2 days before surgery The dogs were kept on cortisone injections for 5 days then maintained on intraperitoneal infusions[5] and utilized for the present experiment from 11 to 14 days after operation Five normal dogs served as control animals

Plasma pepsinogen was studied in dogs before and after stimulation by ACTH and by histamine

ACTH was given subcutaneously in a dose of 5 mg per kg of body weight daily for 2 days Blood for plasma pepsinogen was taken immediately before the first injection 24 hours after each injection and 120 hours after the last injection The test was done after a control period varying from 3 to 24 days (Table 1)

procedure while the mean free acid increased from 10 to 107 clinical units The concentration of pepsin nitrogen per ml increased from 1 35 to 2 20 mg

The experimental data suggest that interruption of the direct neurovascular connections between the hypothalamus and the pituitary gland influences gastric secretion by a resulting deficiency in the release of antidiuretic hormone The efficacy of pitressin administration in restoring the level of the gastric secretory volume reduced by pituitary stalk section supports this view The significant depression of free gastric acidity after administration of pitressin is interesting in view of the effectiveness of pitressin therapy in gastric hyperchlorhydria claimed by Metz and Lackey[5]

Insulin sensitivity as measured by equivalent degrees of hypoglycemia has been found to increase by about threefold in the pituitary stalk sectioned animals Present studies indicate that section of the pituitary stalk and accompanying hypophyseal portal vessels with placement of a barrier does not significantly influence the gastric secretory constituents during the first 5 hours following the induction of insulin hypoglycemia Further investigations will be required to determine by which pathway the adrenocorticotropin release in response to insulin hypoglycemia, which appears to stimulate gastric acid and pepsin secretion is mediated to the pituitary

REFERENCES

1 French J D Longmire R L Porter R W and Movius H J Extravagal influences on gastric hydrochloric acid secretion induced by stress stimuli Surgery *34* 621 1953
2 Shay H and Sun D E Stress and gastric secretion in man I A study of the mechanisms involved in insulin hypoglycemia Am J M Soc *228* 630 1954
3 Harris G W and Jacobsohn D Functional grafts of the anterior pituitary gland Proc. R Soc. Ser B Biol Sc Lond *138*:263 1952
4 Rothballer A B and Skoryna S C Pituitary stalk section in dogs Ann Surg (In Press)
5 Metz M H and Lackey R W Peptic ulcer treated by posterior pituitary preparations Am J Digest Dis 7 27 1940

EFFECT OF ACTH AND HISTAMINE ON PLASMA PEPSINOGEN IN NORMAL HYPOPHYSECTOMIZED THYROIDECTOMIZED AND ADRENALECTOMIZED DOGS*

K KOWALEWSKI H A HYDE AND S T NORVELL JR

Evidence in support of an adrenal phase of gastric secretion has recently been presented It has been shown that various stressor agents as well as the administration of ACTH and adrenal corticoids stimulate the exocrine and endocrine function of gastric cells[3] This stimulation resulting in the secretion of free hydrochloric acid and pepsin with concomitant increase of urinary pepsinogen is independent of the vagi or the gastric antrum[3]

There appears to be an analogy in the response of the stomach to ACTH or stress and to histamine Histamine was shown to be an adequate secretory stimulant of gastric pepsin plasma pepsinogen and uropepsin[7 8] The

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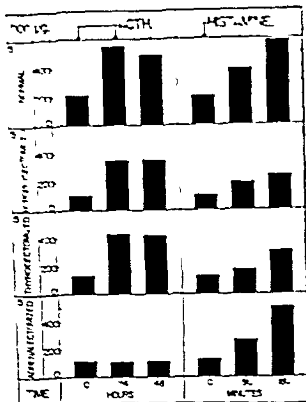


Fig. 1 Comparison of effect of ACTH and histamine on plasma pepsinogen in dogs. Enzyme expressed in micrograms per ml of plasma. Mean values related to Table I

responded better than hypophysectomized and thyroidectomized animals but the most marked increase of plasma enzyme was observed in adrenalectomized dogs. Four of the adrenalectomized animals died in about 8 to 10 hours and one in 24 hours after histamine and the autopsy showed severe congestion and erosions of the gastric mucosa of the stomach. Such findings suggest an increased sensitivity to the injection of histamine. No other animal used in the histamine test showed any signs of systemic or gastrointestinal distress.

Figure 1 gives a comparison of the effect of ACTH and histamine on the gastric endocrine function.

DISCUSSION

Administration of ACTH resulted in a significant increase of plasma pepsinogen in normal hypophysectomized and thyroidectomized dogs. There was no change in plasma enzyme in adrenalectomized dogs treated with ACTH. ACTH is an adequate stimulant of gastric endocrine function and its effect is mediated by the adrenal cortex. The mode of action of cortical steroids on the gastric mucosa is not explained by this experiment. The possibility of the liberation or activation of endogenous histamine following pituitary adrenal stimulation can be considered seriously.

Exogenous histamine is an adequate stimulant for production of pepsinogen. Hypophysectomy did not abolish the posthistaminic increase of plasma pepsinogen. This observation supports the view[4] that histamine is not a stressor factor acting necessarily through the release of ACTH. The release of ACTH from the pituitary following the administration of histamine noted by other authors[1,7,11] may not have the physiologic importance attributed to it. Thyroidectomized dogs responded to histamine by marked but slightly delayed reaction. Adrenalectomy reduced the tolerance to histamine which is in agreement with the work of other authors.[10]

The histamine test was performed 2 weeks after the ACTH test Adrenalectomized dogs however received histamine 120 hours after the last injection of ACTH The dogs were protected against the systemic effects of histamine by intramuscular injection of an antihistaminic Phenergan (Poulenc[8]) Blood was taken immediately before at 90 minutes and at 180 minutes after the subcutaneous injection of histamine dihydrochloride (Roche) Both phenergan and histamine were given in a dosage of 5 mg per kg of body weight

Assay of the plasma pepsinogen was carried out following the modified[3] method of Mirsky [9] Results are expressed in terms of micrograms of tyrosine released by the proteolytic action of one ml of plasma

RESULTS

The results of ACTH test are given in Table 1 Response to the stimulation with ACTH is marked in all but adrenalectomized dogs It is apparent from this table that in the absence of adrenal glands ACTH does not stimulate the endocrine function of zymogenic cells of the stomach

Table 1 also gives the results of the histamine test It can be noticed from the reported data that all animals responded to the administration of histamine by a significant increase of plasma pepsinogen Control dogs

Table 1 Effect of ACTH and histamine on plasma pepsinogen of dogs in various endocrine conditions Sampling of blood in ACTH test before injection (0) 24 hours after each dose of ACTH (I) and (II) and 120 hours after the last injection (III) Sampling of blood for histamine test before injection (0) and 90 and 180 minutes after histamine

		CONTROL* PERIOD	ACTH TEST					HISTAMINE TEST				
CONDITION	DOG	MEAN S D	0	I	II	III	RISE IN % IN 24 HRS	0	90	180	RISE IN % IN 90	RISE IN % IN 180 MIN
Normal	1	234±31	251	565	580	210	125	202	350	540	73	167
	2	208±19	183	603	459	261	229	201	430	580	114	188
	3	193±16	191	524	418	257	174	185	336	534	81	188
	4	210±23	230	516	452	240	124	193	323	590	67	205
	5	234±25	220	543	540	258	147	258	494	686	91	166
Hypophysectomized	6	117± 8	125	380	313	128	204	124	190	272	53	119
	7	104±12	94	345	387	134	267	96	184	219	92	128
	8	112± 9	122	343	378	106	181	110	186	238	69	116
	9	92±13	82	291	275	181	254	96	190	202	98	110
	10	108±10	107	401	442	112	278	133	216	287	62	116
Thyroidectomized	11	147±19	150	507	501	239	238	118	179	280	51	136
	12	146±17	120	397	391	211	214	139	187	329	35	136
	13	142±12	152	448	410	220	195	141	160	327	13	132
	14	130± 8	139	444	386	212	219	140	159	321	13	129
	15	140±11	129	371	414	247	187	135	205	308	52	128
Adrenalectomized	16	135 †	132	130	138	152	0	152	342	594	125	291
	17	160 †	154	154	158	144	0	144	337	445	134	209
	18	67 †	63	57	68	72	0	72	162	288	125	300
	19	136 †	140	140	139	142	0	162	316	718	95	342
	20	92 †	99	86	93	89	0	89	205	458	130	414

*Control period of 24 days with 7 samples taken every fourth day

†Control period of 3 days preceding the test sampling every day

THE EFFECT OF RESECTION OF THE DUODENUM ON GASTRIC SECRETION OF HYDROCHLORIC ACID*

Edwin L Brackney, Alan P Thal and Owen H Wangensteen

The fact that the duodenum may have an important role in the control of gastric secretory activity has been recognized for many years Szokolow[1] showed that both fat and HCl introduced into the duodenum through a duodenal fistula would cause a marked inhibition in the rate of secretion of HCl by isolated gastric pouches An indication that exclusion of the contents of the stomach from contact with the duodenal mucosa might upset the balance of gastric secretion was furnished by Storer Oberhelman Woodward Smith and Dragstedt[2] They found that, in dogs with gastric pouches, the Exalto-Mann Williamson operation was regularly followed by a marked increase in hydrochloric acid secretion

The experiments reported here were undertaken in an attempt to demonstrate whether this effect might be due chiefly to loss of inhibitory effect of the duodenum on the gastric secretion

METHOD

Mongrel dogs with isolated fundic (Heidenhain) pouches were used in these experiments After a first operation in which the isolated fundic pouch was made the animal was allowed to recover for 2 or 3 weeks and then the HCl secretion of the pouch was standardized This was done by keeping a record of the total amount of HCl secreted by the pouch over a standard 8 hour test period 3 days a week for 4 or 5 weeks The dogs were fasted for 16 hours prior to each collection and were given a standard 200 gm cooked horse meat meal after the first hour of the collection period The amount of HCl secreted during the test period in mEq was calculated by multiplying the free acidity in clinical units by the volume of secretion in liters

After the pouches were standardized the duodenum and proximal 20 to 30 cc of jejunum were resected and discarded A cuff of duodenum about 2 cm long was left attached to the pylorus to preserve the function of the pyloric sphincter and prevent abnormal regurgitation of intestinal content back into the stomach The distal cut end of the jejunum was anastomosed end to end to the remnant of duodenum attached to the stomach The common bile duct and the main pancreatic duct were anastomosed end to-side to the jejunum in approximately their normal relationship to the pylorus by direct mucosa to mucosa suture with interrupted 6 0 silk

The dogs were allowed to recover from their second operation for a week or more and then the HCl secretion of their isolated fundic pouches was again standardized

RESULTS

There was a marked increase in the amount of HCl secreted by the isolated fundic pouches of all of the animals This increase ranged from 84 per cent to 820 per cent in the 5 animals Table 1 summarizes the data on these animals and Figure 2 is the graph of the response of a typical dog to duodenal resection

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*From the Department of Surgery University of Minnesota Medical School Minneapolis Minn Supported by United States Public Service Grant No RG 1028 (C7) Studies in The Etiology of Acid peptic Ulcer

It was suggested[10] that the reduction of tissue histaminase in the adrenalectomized animals may be responsible for the lack of resistance to both endogenous and exogenous histamine This hypothesis may explain the presence of gastric lesions and very marked increase of pepsinogen in our adrenalectomized animals Histamine appears to be an adequate stimulant of endocrine function of the gastric mucosa in dogs, acting probably directly on zymogenic cells even in the absence of the pituitary thyroid or adrenals

SUMMARY

1 Administration of ACTH resulted in marked increase of plasma pepsinogen in normal, hypophysectomized and thyroidectomized dogs There was no change in plasma pepsinogen in adrenalectomized animals treated with ACTH

2 Administration of histamine resulted in marked increase of plasma pepsinogen in normal hypophysectomized thyroidectomized and adrenalectomized dogs It is apparent from this part of the experiment that histamine is not a stressor factor acting necessarily through the release of ACTH

3 Absence of adrenals increased the sensitivity of animals to histamine The increase of plasma pepsinogen in adrenalectomized dogs was much higher than in normal controls

We are indebted to Dr T C Speakman from the Department of Neurosurgery for performing the hypophysectomy in our dogs and to Dr T Shnitka from the Department of Pathology of the University of Alberta for reading the histological preparations

REFERENCES

1 Chi Ping Cheng Sayers G Goodman L S and Swinyard Ch A Discharge of adrenocorticotrophic hormone in the absence of neural connections between the pituitary and hypothalamus Am J Physiol *158* 45 50 1949
2 Chi Ping Cheng Sayers G Goodman L S and Swinyard Ch A Discharge of adrenocorticotrophic hormone from transplanted pituitary tissue Am J Physiol *159* 426 432 1949
3 Grey Y S Ramsay C Reifenstein R W and Benson J A The significance of hormonal factors in the pathogenesis of peptic ulcer Gastroenterology *25* 156 172 1953
4 Guillemin R A re evaluation of acetylcholine adrenaline nor adrenaline and histamine as possible mediators of the pituitary adrenocorticotrophic activation by stress Endocrinology *56* 248 255 1955
5 Grollman A The maintenance of the adrenalectomized dog for prolonged periods without recourse to hormonal therapy Endocrinology *50* 331 337 1952
6 Kowalewski K and Bain C O Prevention of post histaminic gastric ulcers in guinea pigs by posterior pituitary extract Acta gastroenter Belg *17* 539 551 1954
7 Kowalewski K Uropepsin and plasma pepsinogen after the injection of histamine dihydrochloride in doses provoking acute gastric ulcers in guinea pigs Canad J Bioch Physiol *32* 553 558 1954
8 Kowalewski K and Norvell S T Jr Relationship between dose and response in posthistaminic plasma pepsinogen in dogs Canad J Bioch Physiol *33* 599 601 1955
9 Mirsky A Futterman P Kaplan S and Brohkahn R H Blood plasma pepsinogen The source properties and assay of the proteolytic activity of plasma at acid reactions J Laborat Clin M *40* 17 26 1952
10 Rose B and Browne J S L The effect of adrenalectomy on the histamine content of the tissues of the rat Am J Physiol *131* 589 594 1941

Table 1. Mean Output of Free HCl by Isolated Gastric Pouches Before and After Resection of the Duodenum and Proximal 30 cm. of Jejunum. (The difference between the preoperative and postoperative means is statistically significant in every case.)

DOG NO.	MEAN OUTPUT OF HCl PER 8 HR. COLLECTION PERIOD IN MEQ.		PER CENT INCREASE FOLLOWING OPERATION
	PREOPERATIVE	POSTOPERATIVE	
1	1.54	4.51	194
2	1.60	4.27	168
3	.29	2.67	820
4	5.79	10.63	84
5	4.01	11.32	182

DISCUSSION

These experiments show clearly that resection of the duodenum in the dog is accompanied by a marked increase in the amount of hydrochloric acid secreted by the stomach. Three different lines of evidence seem to indicate that this increase is due to the loss of an inhibitory effect of the duodenum on gastric secretion. This inhibitory effect is probably brought about when the gastric contents pass into and through the duodenum. First, the studies of Szokolow[1] and Day and Komorov[3] have shown that the presence of fat, HCl, gastric juice, or sugars in the duodenum inhibits secretion of HCl by the stomach; second, when gastric content is excluded from the duodenum in the Exalto-Mann-Williamson operation as reported by Storer *et al.*[2] there is a marked increase in the output of HCl by isolated gastric pouches; and third, it was found in this laboratory[4] that interposition of a long segment of small bowel between the stomach and duodenum, so that the gastric content entered the duodenum only after it had been acted upon by the intestine, resulted in a marked increase in the output of HCl by isolated gastric pouches.

If this interpretation of these results is correct, then it may serve to emphasize the importance of maintaining gastroduodenal continuity in the design of gastric resections for peptic ulcer.

SUMMARY

Resection of the duodenum and proximal 20 cm. of jejunum results in a marked increase in the secretion of hydrochloric acid by isolated gastric (Heidenhain) pouches. It is possible that this effect is due to the loss of an inhibitory control mechanism exercised by the duodenum over gastric secretion. The nature of this control mechanism is not clear at this time.

REFERENCES

1. Szokolow, A.: Zur analyse der Abscheidungsarbeit des Magens bei Hunden. Jahrber. Fortsch. Tierchem. *34*: 469-470, 1904.
2. Storer, E. H., Oberhelman, H. A., Woodward, E. R., Smith, C. A., and Dragstedt, L. R.: The effect of the Exalto-Mann-Williamson procedure on gastric secretion. Arch. Surg. *64*: 192-199, 1952.
3. Day, J. J., and Komorov, S. A.: Glucose and gastric secretion. Am. J. Digest. Dis. *6*: 169, 1939.
4. Brackney, E. L., Thal, A. P., and Wangensteen, O. H.: Role of duodenum in the control of gastric secretion. Proc. Soc. Exp. Biol. N. Y. *88*: 302-306, 1955.

Fig 1 Resection of the duodenum and proximal 20 to 30 cm of jejunum End to end anastomosis of jejunum to duodenal remnant on stomach End to side suture anastomosis of common bile duct and main pancreatic duct to jejunum

Fig 2 Effect of resection of the duodenum and proximal jejunum on secretion of HCl by a Heidenhain Pouch

stand quietly in a loosely restraining dog sling. A cardiac catheter for sampling of mixed venous blood was passed into the pulmonary artery under general anesthesia 1 to 10 days previous to the time of the experiment and left in situ with the peripheral end of the catheter buried beneath the skin. Two or 3 hours before the experimental period this superficial end of the cardiac catheter was exposed and a second indwelling catheter introduced under local anesthesia into the femoral artery for the collection of arterial blood. Cardiac output was calculated according to the direct Fick principle utilizing oxygen consumption and arterial-venous differences of oxygen. The methods for collection of respiratory gases and the chemical analysis were carried out according to methods described elsewhere.[4]

RESULTS

Table 1 illustrates 2 typical experiments carried out on the same animal. As shown, the intrajejunal administration of either hypertonic saline (9 per cent) or glucose (50 per cent) in quantitatively similar amounts resulted in a decrease in cardiac output of 28 and 35 per cent and stroke volume of 18 and 39 per cent.

Table 2 gives the data on 2 experiments on an animal with jejunal "button" and compares the effects of hypertonic glucose when given (1) directly into the stomach and (2) directly into the jejunum. As noted here there was a marked decrease in cardiac output when the glucose was

Table 1

DATE	4/20 C.O (L./MIN.)	4/20 S.V (CC.)	4/21 C.O (L./MIN.)	4/21 S.V (CC.)
Control	4.50	38	4.45	39
Control	4.85	44	4.12	35
	150 cc. 50% Glucose		150 cc. 9% NaCl	
20 min	3.95	25	3.46	33
40 min	3.23	20	2.90	24

Solutions of 50 per cent glucose and 9 per cent NaCl administered per jejunum to the same dog produced similar decreases in cardiac output (—28 per cent and —35 per cent). Stroke volume fell from 38 cc. to 20 cc. (—48 per cent) on April 20 and from 39 cc. to 24 cc. (—39 per cent) in experiment on April 21.

Table 2

DOG NO. 195 (STOMACH INTACT, JEJUNAL BUTTON)

	CARDIAC OUTPUT	CARDIAC OUTPUT
Control	3.28	3.14
Control	2.91	3.03
	150 cc. 50% glucose via Jejunum	150 cc. 50% glucose via Stomach
20 min	2.08 (—37%)	2.83 (—10%)
40 min	2.12 (—35%)	3.41 (+ 9%)

50 per cent glucose solution placed into the jejunum reduced the cardiac output. The same solution given via stomach did not alter it significantly at 20 or 40 minutes. Experiments conducted on different days.

CHANGES IN CARDIAC OUTPUT DURING THE DUMPING SYNDROME*

LEO D KLAUBER J WILLIAM POPPELL, HENRY T RANDALL AND KATHLEEN E ROBERTS

The signs and symptoms of the dumping syndrome may be invoked when hypertonic solutions are introduced into the jejunum or when ingested hypotonic foods are made hypertonic in the course of enzymatic breakdown in the jejunum[1] It has been postulated that hypertonic solution induces an acute shift of extracellular water into the intestinal lumen The resultant decrease in circulating blood volume may be accompanied by a drop in blood pressure tachycardia and electrocardiographic changes suggestive of myocardial ischemia The sympathetic manifestations[3] of the dumping syndrome have been assumed to be secondary to the decrease in blood volume and a part of the compensatory mechanisms involved in maintaining cardiac output However the decrease in blood pressure and the electrocardiographic changes suggest that cardiac output is not completely maintained in the patients with severe dumping symptoms[4] The experiments reported here were carried out on dogs in an attempt to evaluate the extent of changes in cardiac output which may accompany the dumping syndrome as precipitated by intrajejunal administration of hypertonic glucose or saline

The results of these studies indicate that there is a consistent decrease in both cardiac output and stroke volume following the administration of these solutions into the jejunum

METHOD

Since it has been shown that the intrajejunal administration of hypertonic solutions may precipitate a similar sequence of events in both dog and man the studies reported here were carried out utilizing the intrajejunal administration of hypertonic glucose or saline[5] In all a total of 12 experiments were carried out on 8 dogs Three groups of animals were used for these studies (1) normal dogs (2) gastrectomized dogs in which the hypertonic solutions were given by Levine tube and (3) dogs with intact stomachs in which the hypertonic solution was introduced directly into the jejunum through a permanent fistula created by the insertion of a steel tube flanged at both ends which communicated directly from the skin to the intestinal lumen When not in use the opening of the steel button was closed by a center screw

Three measurements of cardiac output and stroke volume were carried out during the control period The animals were then given 75 to 150 cc of hypertonic glucose or saline and cardiac output measured at intervals of 20 and 40 minutes following the administration of hypertonic solution All experiments were performed on fasting unanesthetized animals trained to

*From the Division of Experimental Surgery The Andre and Bella Meyer Physiology Laboratory of the Sloan Kettering Institute and the Departments of Surgery and Medicine Memorial Center New York N Y and the Sloan Kettering Division of Cornell University Medical College New York N Y Supported by U S P H S grant number CS9261 C

We are indebted to Dr J M Walker for his interest and cooperation in carrying out this work and to Serge Deneeko Margaret Hood and Jeanette Louzon for their invaluable technical assistance

to express these changes statistically The direction of change however was consistent in all animals

Presumably the decrease in blood volume which has been shown to occur following the intrajejunal administration of hypertonic foods would also result in a decrease in stroke volume Although tachycardia is a consistent finding during the dumping episode in man and was also noted in the animals reported here the decrease in stroke volume may be of sufficient magnitude that cardiac output is not maintained Such was found to be the case in the animals studied by us Furthermore these findings suggest that the failure of some patients to maintain blood pressure during a severe dumping episode may result in a decreased cardiac output and that the symptoms and electrocardiographic changes may be related in part to decreased coronary blood flow Support is given to this reasoning by the recent findings of Katz *et al*[7] who showed that coronary blood flow may be reduced when cardiac output is lowered and blood pressure not maintained If this supposition is correct it suggests that a decreased cardiac output and possibly a decrease in coronary flow with resultant myocardial ischemia, may contribute to the severity of the dumping syndrome especially in patients with pre existing myocardial damage or in patients with an already contracted blood volume

CONCLUSIONS

Hypertonic solutions of glucose or saline when given by Levine tube to gastrectomized dogs or intrajejunally in otherwise intact animals result in a decrease in cardiac output and stroke volume It is suggested that the decreased cardiac output consequent to a decrease in blood volume in part may account for the symptoms and electrocardiographic findings observed in the dumping syndrome

REFERENCES

1 Machella T F The mechanism of the post gastrectomy dumping syndrome Ann Surg *130* 145 1949
2 Roberts K F Randall H T and Farr H W Acute alterations in blood volume plasma electrolytes and electrocardiograms produced by the oral administration of hypertonic solutions to gastrectomized patients in Surgical Forum 1953 Philadelphia W B Saunders Co 1954 301 306
3 Fulton John F Regulation of Arterial Pressure A Textbook of Physiology 16th Ed Philadelphia W B Saunders Co 1950 pp 750 755
4 Roberts K E Randall H T Bane H N Medwid A and Schwartz M K Studies of the physiology of the dumping syndrome N Y State J M (In press)
5 Joly D and Bane H N Personal communication
6 Roberts K E Poppell J W Vanamee P Beals R and Randall H T Evaluation of respiratory compensations in metabolic alkalosis J Clin Invest (In press)
7 Katz A M Katz L N Williams F L Regulation of coronary flow Am J Physiol *180* 392 1955

Table 3

DATE	DOG	PROCEDURE	SOLUTION USED	CARDIAC OUTPUT (MAXIMAL CHANGE)	STROKE VOLUME (MAXIMAL CHANGE)
4/4	65	Jejunal Button	75 cc 50% Gluc	—19%	—35%
4/17	169	Jejunal Button	75 cc 50% Gluc	—19%	—21%
4/12	147	Gastrectomy	100 cc 50% Gluc	—15%	—68%
4/13	147	Gastrectomy	100 cc 9% NaCl	—11%	—51%
4/28	147	Gastrectomy	100 cc 9% NaCl	—37%	—3[illegible]%
4/11	10[illegible]	Jejunal Button	100 cc 50% Gluc	—18%	—26%
4/19	90	Gastrectomy	100 cc 50% Gluc	—30%	—45%
4/20	209	Jejunal Button	150 cc 50% Gluc	—28%	—48%
4/21	209	Jejunal Button	150 cc 9% NaCl	—3[illegible]%	—39%
4/27	19[illegible]	Jejunal Button	150 cc 50 [illegible] Gluc	—37%	—31%

Following the administration of hypertonic glucose or saline the decrease in cardiac output and stroke volume is evident in all animals regardless of whether the stomach was intact and the solutions given through the intrajejunal button or the animal was gastrectomized and the substance given by Levine tube. Both hypertonic glucose and saline produced similar results. The change recorded is the maximal one found at either the 20 or 40 minute period. Dog number 90 died with pulmonary edema after the experiment of April 19.

given intrajejunally, and insignificant changes were noted when the same substance was given via the stomach.

As summarized in Table 3 changes in cardiac output and stroke volume were evident in all animals following the intrajejunal administration of hypertonic solutions regardless of whether the stomach was intact and the solutions given through the intrajejunal button, or whether the animal was gastrectomized and the substance given by Levine tube. Furthermore, it was noted that hypertonic glucose or saline produced quantitatively similar results.

Clinically, the administration of hypertonic solutions often resulted in marked weakness of the animals and a barely perceptible pulse. Of some interest was our observation that the animals that were subjected to total gastrectomy and had experienced considerable weight loss were found to have lower cardiac outputs during the control period than the animals whose stomachs were intact. One of the gastrectomized dogs died within 2 hours of the experiment as the result of pulmonary edema.

DISCUSSION

From the data shown it is apparent that the intrajejunal administration of hypertonic solutions may result in a profound decrease in cardiac output and stroke volume. The number of animals studied is not sufficient

to express these changes statistically The direction of change, however was consistent in all animals

Presumably the decrease in blood volume which has been shown to occur following the intrajejunal administration of hypertonic foods would also result in a decrease in stroke volume Although tachycardia is a consistent finding during the dumping episode in man and was also noted in the animals reported here the decrease in stroke volume may be of sufficient magnitude that cardiac output is not maintained Such was found to be the case in the animals studied by us Furthermore these findings suggest that the failure of some patients to maintain blood pressure during a severe dumping episode may result in a decreased cardiac output and that the symptoms and electrocardiographic changes may be related in part to decreased coronary blood flow Support is given to this reasoning by the recent findings of Katz *et al*[7] who showed that coronary blood flow may be reduced when cardiac output is lowered and blood pressure not maintained If this supposition is correct it suggests that a decreased cardiac output and possibly a decrease in coronary flow with resultant myocardial ischemia may contribute to the severity of the dumping syndrome especially in patients with pre existing myocardial damage or in patients with an already contracted blood volume

CONCLUSIONS

Hypertonic solutions of glucose or saline when given by Levine tube to gastrectomized dogs or intrajejunally in otherwise intact animals result in a decrease in cardiac output and stroke volume It is suggested that the decreased cardiac output consequent to a decrease in blood volume in part may account for the symptoms and electrocardiographic findings observed in the dumping syndrome

REFERENCES

1 Machella T F The mechanism of the post gastrectomy dumping syndrome Ann Surg *130* 145 1949
2 Roberts K F Randall H T and Farr H W Acute alterations in blood volume plasma electrolytes and electrocardiograms produced by the oral administration of hypertonic solutions to gastrectomized patients in Surgical Forum 1953 Philadelphia W B Saunders Co 1954 301 306
3 Fulton John F Regulation of Arterial Pressure A Textbook of Physiology 16th Ed Philadelphia W B Saunders Co 1950 pp 750 755
4 Roberts K F Randall H T Bane H N Medwid A and Schwartz M K Studies of the physiology of the dumping syndrome N Y State J M (In press)
5 Joly D and Bane H N Personal communication
6 Roberts K E Poppell J W Vanamee P Beals R and Randall H T Evaluation of respiratory compensations in metabolic alkalosis J Clin Invest (In press)
7 Katz A M Katz L N Williams F L Regulation of coronary flow Am J Physiol *180* 392 1955

THE VALUE OF FINNEY PYLOROPLASTY IN MINIMIZING ESOPHAGITIS AFTER ESOPHAGOGASTRECTOMY WITH VAGOTOMY AND ESOPHAGOGASTROSTOMY

*AN EXPERIMENTAL STUDY IN DOGS**

George W Girvin and K Alvin Merendino

Clinical experience with esophagogastrostomy following resection of the cardiac sphincter in the treatment of various diseases of the lower esophagus and proximal stomach has been unsatisfactory This is apparently true regardless of the position of the anastomosis with regard to the level of the diaphragm Experimental studies have shown that resection of the cardiac sphincteric mechanism together with bilateral vagotomy combined with approximately 50 per cent of the total proximal stomach results in the spontaneous development of esophageal ulcers in approximately half of the animals[1] Of this series 6 animals were subjected in addition to a pyloromyotomy Three of these animals had severe esophageal pathology however, the remaining animals were alive and well without evidence of complications at the end of 6 months

A follow up study with an identical experimental preparation was carried out with additional supplementary gastric drainage procedures In those animal with gastrojejunostomy esophageal ulcer some with perforation and bleeding occurred in all 5 dogs Therefore it was felt that an ancillary drainage procedure supplementing the experimental preparation *described above did not affect the development of esophagitis* and its complications A more critical study then was carried out in which a series of animals was subjected to 50 75 and 100 per cent resection of the functioning stomach**[3] These animals in addition were subjected to a bilateral vagotomy but all had intact pyloric sphincters Under chronic histamine stimulation esophagitis appeared universally even in those subjected to almost 100 per cent excision of the functioning stomach This result undoubtedly was due in part to the secondary effects of vagotomy viz gastric atony and pylorospasm which resulted in a prolongation of the antral phase of gastric secretion More recently in Heidenhain pouch dogs it has been demonstrated that a Finney pyloroplasty restored pouch secretion to normal following the usual increased secretion after vagotomy[4] Additional supportive evidence has been gained in the use of vagotomy and Finney pyloroplasty when a jejunal segment is interposed between the esophagus and the stomach Vagotomy and Finney pyloroplasty in spite of chronic histamine stimulation protected not only the stomach and duodenum but also the interposed jejunal segment[5]

Recent work in this laboratory has indicated that a pyloromyotomy is inadequate as a drainage procedure for the vagotomized stomach In addition the circus movements attributable to gastrojejunostomy may accentuate

*From the Department of Surgery University of Washington School of Medicine Seattle Wash Supported in part by Public Health Grant #C2186 and by Initiative 171 Funds from the State of Washington for Research in Biology and Medicine

** Functioning stomach refers to that portion concerned mainly with the secretion of acid and pepsin In this frame of reference a 100 per cent excision of the functioning stomach would indicate a resection of the stomach from the esophagogastric junction down to the anatomic antrum

the hormonal (antral) phase rather than reduce it.[4] Consequently these experiments have been designed to test the effects of combined vagotomy and Finney pyloroplasty after esophagogastrectomy and esophagogastrostomy with particular regard to the effect of pyloroplasty on the development of esophagitis.

METHOD

Seventeen mongrel dogs weighing between 7 to 11 kg. were used in these experiments.

Operative Procedure. Under nembutal anesthesia by means of a midline abdominal incision each animal was subjected to an esophagogastrectomy, a bilateral vagotomy and a Finney pyloroplasty. The esophagus was uniformly transected 1 cm. proximal to the esophagogastric junction. The series developed for study varied only in the extent of resection of the functioning stomach. Consequently 4 series of animals were created, namely those subjected to 25, 50, 75 and 100 per cent resection of the functioning stomach.

Continuity of the gastrointestinal tract was established by esophagogastrostomy along the lesser curvature. The anastomosis was always retained beneath the diaphragm. No diaphragmatic incision was made in any animal. All pyloroplasties were 4 cm. in length. In addition a cottonoid pattern of the resected portion of the stomach as well as the remnant proximal to the arteries was made in order to establish the per cent of resection. Area measurements were made by planimeter.[6] Consequently the classification of animals in the various series is based on the actual percentile resection carried out.

Postoperatively food and water were withheld for 3 days and 500 cc. of 5 per cent dextrose in saline per 10 kg. weight were given. Water was allowed by mouth on the fourth day and a purée diet of milk and Friskies was added by the seventh day. A general diet was resumed in 2 weeks.

Other studies. Preoperatively and postoperatively the average secretory response to acqueous histamine was obtained. By gastric aspiration a fasting basal sample was obtained. Twenty minutes following the subcutaneous administration of 0.5 mg. of histamine diphosphate the stomach was again aspirated as completely as possible. In those animals subjected to a 75 per cent or greater resection no samples were obtainable postoperatively. The volume, free and total acid was determined on all specimens. The average pre- and postoperative histamine response thus was obtained.

Esophagoscopy was carried out on all animals and in the majority on more than one occasion. Chronic histamine stimulation was begun on the average 90 days postoperatively. Each animal received 30 mg. of histamine base in beeswax daily for 30 days. At the time of sacrifice gross and microscopic examination was carried out. Representative sections were taken where indicated.

RESULTS

All animals survived at least 4 weeks of chronic histamine stimulation. Two of them appeared moribund but the remainder seemed healthy when sacrificed. Table 1 compares the results of the 4 groups of proximal gastrectomies.

Table 1 Esophagogastrectomy with Esophagogastrostomy Combined with Vagotomy and Finney Pyloroplasty

				Esophagoscopy		Gastric Analyses: Preoperative			Gastric Analyses: Postoperative*			Autopsy Findings†		
Dog No	Area Resected	Area Retained	Per Cent Resected	Days	Results	Vol	Free	Total	Vol	Free	Total	Esophagus	Stomach	Duodenum
X 71	21.4	66.3	24	75 & 106	Red 2 cm prox	16	52	67	12	10	13	1	3	3-4
X 72	20.8	62.7	23	74 & 105	No reddening	13	73	82	11	23	36	0	0	0
X 73	19.2	58.1	25	70 & 101	Red 1 cm prox	24	37	50	12	18	38	0	3°	0
X 75	22.0	68.4	24	69 & 100	Red 1 cm prox	15	23	32	10	12	20	1°	3	1°
X 77	25.0	20.6	54	61 & 92	Red 2 cm prox	10	65	77	8	22	30	1	1	1
X 78	42.0	31.9	56	61 & 92	Red 1 cm prox	17	47	58	15	32	53	0	0°	0
X 80	51.2	48.2	52	60 & 91	Red 2 cm prox	20	66	81	13	16	33	1°	2°	4
X 84	34.1	33.8	50	59 & 90	Red 2 cm prox	15	75	92	21	26	38	1	2°	1
X 89	48.6	17.7	72	60	Red 1 cm prox	17	65	78	No Fluid Obtainable			0	0	0°
X 62	52.8	18.4	74	72	Red 1 cm prox	18	43	61	No Fluid Obtainable			0	2	0°
X 97	50.7	20.1	74	56	Red 2 cm prox	15	50	62	No Fluid Obtainable			1°	0	0
X 96	44.7	15.2	74	32	No reddening	18	66	79	No Fluid Obtainable			1°	2	0
X 86	73.8	0	100	73	No reddening	22	45	61	No Fluid Obtainable			0	0	0
X 88	41.9	0	100	68	No reddening	16	31	43	No Fluid Obtainable			0	0	0°
X 79	68.6	0	100	69	No reddening	39	88	101	No Fluid Obtainable			0	0	0
X 95	62.9	0	100	32	No reddening	21	30	42	No Fluid Obtainable			0	0°	0

*Average response to aqueous histamine stimulation

†1 Inflammation only

2 Erosion not greater than 1 cm in diameter and not extending into muscularis

3 Erosion greater than 1 cm in diameter or ulcer extending into muscularis

4 Perforation

First of all there were no esophageal ulcers seen in any of the animals either by esophagoscopy or at sacrifice. By esophagoscopy 10 of 16 animals showed some reddening of the lower esophagus, however, without stenosis. At autopsy about 50 per cent in each the 25, 50 and 75 per cent resection groups showed reddening of the esophageal mucosa. This was usually within 1 or 2 cm. proximal to the anastomosis. The mucosa was intact on microscopy although there was some leukocytic infiltration mainly by lymphocytes. There was no inflammation in the group having 100 per cent resections of the "functioning" stomach (see Figs. 1 and 2).



Fig. 1. Dog number X 71. This animal was subjected to a 25 per cent gastric resection. After chronic histamine stimulation a gastric ulcer, perforated duodenal ulcer and 1 esophagitis was demonstrated. While the esophagitis appears severe, the mucosa was intact. Undoubtedly had this animal survived longer a severe esophagitis would have resulted. (See Table 1)

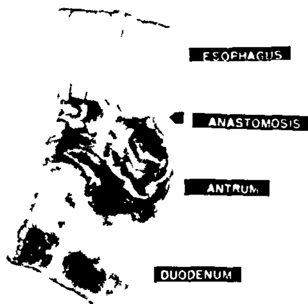


Fig. 2. Dog number X 85. A 100 per cent proximal gastrectomy with esophagoantrostomy. This photograph demonstrates well the total lack of reaction thus emphasizing the importance of the acid peptic factor in problems of lower esophagitis.

Seven animals developed gastroduodenal peptic ulcers on histamine stimulation but only 1 animal in each of the 25 and 50 per cent resection groups had a perforating duodenal ulcer

DISCUSSION

The results indicate that when an esophagogastrectomy and vagotomy are performed and intestinal continuity is restored by esophagogastrostomy the incidence and severity of esophagitis appears to be reduced by the addition of a Finney pyloroplasty It should be stressed that esophagitis is not prevented but rather is minimized

This suggests that in similar experiments with an intact pylorus carried out previously[2] the high incidence of severe esophageal lesions was directly related to the stimulation of the antral phase of gastric secretion secondary to pylorospasm and gastric retention created by vagotomy Unfortunately the period of chronic histamine stimulation in this series was only 30 days whereas in the earlier series a 45 day s period of histamine stimulation was carried out Consequently the stimulating phase was reduced by one third Thus one may question the validity of a direct comparison of these 2 series of studies However esophagoscopic findings in the previous series prior to histamine stimulation indicated a high incidence of esophagitis with some instances of ulcer and stricture already present In the present series esophagoscopic examination at similar periods previous to histamine stimulation while indicating some reddening and therefore esophagitis to be present did not reveal a single ulcer or stricture Therefore in spite of the reduced period of histamine preparation it appears that the addition of an adequate pyloroplasty definitely reduces the incidence and severity of esophagitis

The presence of esophagitis however in these short term experiments indicates the imperfections of the operative procedure as an attack on esophagitis It is well known that in man a prolonged period may be necessary to convert a mild esophagitis into a severe stricture In short alternate periods of irritation and healing with fibrosis progresses slowly over a number of years

In view of the fact that reflux esophagitis is an acid peptic problem it is understandable that the greater the percentile resection of parietal cells the less the incidence of gastroduodenal pathology Only in those animals subjected to 100 per cent resections of the functioning stomach was there a complete absence of esophagitis and gastroduodenal lesions Because of the need for an extensive gastrectomy this procedure would not be recommended for acid peptic disease in this area

The study suggests that in situations where an esophagogastrectomy is to be carried out and acid peptic disease is not the primary indication the addition of a vagotomy and Finney pyloroplasty should reduce the incidence of esophagitis and minimize the symptomatology which not uncommonly follows esophagogastrostomy

CONCLUSIONS

1 Seventeen dogs were subjected to esophagogastrectomy Four series of animals were developed dependent upon the percentile resection of the stomach proximal to the antrum Additional complemental procedures included bilateral vagotomy and Finney pyloroplasty

2 By the addition of a Finney pyloroplasty in the presence of a 100 per cent resection of the functioning stomach esophagitis was prevented

3 Where the proximal gastrectomy varied from 25 through 75 per cent the presence of a Finney pyloroplasty reduced the incidence and severity of esophagitis but did not prevent it

4 This procedure is not advocated for lesions of the lower esophagus of acid peptic origin However in other situations where a vagotomy is combined with esophagogastrectomy and esophagogastrostomy a Finney pyloroplasty appears to be the best ancillary drainage procedure in minimizing the development and severity of esophagitis

REFERENCES

1 Kiriluk, L B and Merendino K A An experimental evaluation in the dog of esophagogastrectomy for the high lying gastric ulcer Ann Surg 134 918 925 1951
2 Kiriluk L B and Merendino K A Further experiences in the dog with esophageal pathology following esophagogastrectomy in Surgical Forum 1951 Philadelphia W B Saunders Co 1952 pp 59-65
3 Hoag E W, Kiriluk L B and Merendino K A Experiences with upper gastrectomy Its relationship to esophagitis with special reference to esophagogastric junction and the diaphragm Am J Surg 88 44 55 1954
4 Nyhus L M, Kanar E A, Moore H G, Jr Sauvage L R Schmitz E J Storer E H and Harkins H N Gastrojejunostomy and Finney pyloroplasty their effects upon Heidenhain pouch secretion in vagotomized and non vagotomized dogs in Surgical Forum 1952 Philadelphia W B Saunders Co 1953 p 316
5 Dillard D H and Merendino K A Experiences with the interposed jejunal segment operation combined with adjunct procedures in the prevention of esophagitis in Surgical Forum 1953 Philadelphia W B Saunders Co 1954 pp 323 328
6 Moore H S, Jr, and Harkins H N A critical evaluation of the Billroth I gastric resection Surgery 32 407 425 1952

THE IMPORTANCE OF ESOPHAGODUODENAL CONTINUITY FOLLOWING TOTAL GASTRECTOMY*

FREDERICK M BINKLEY HAROLD A HARPER AND
HORACE J MCCORKLE

Total gastrectomies have been performed on 131 dogs in this laboratory and intestinal continuity has been re established by esophagoduodenostomy esophagojejunostomy or by the transposition of a jejunal or colon segment between the esophagus and duodenum Postoperatively these animals have been evaluated for periods up to 4 years with investigation of protein carbohydrate and fat metabolism at varying intervals Observations of the animals general appearance weight and metabolism has indicated that the nutrition of the animals in which esophagoduodenal continuity was maintained was superior to that of those animals in which the duodenum was bypassed[1 2 3 4 5] In this investigation nitrogen balance studies dem

*From the Surgical Research Laboratories of the University of California School of Medicine San Francisco Calif Supported by Cancer Research Funds of the University of California

onstrated less fecal nitrogen excretion in those animals in which the duodenum was not bypassed as follows

RECONSTRUCTIVE PROCEDURE	PERCENTAGE OF INGESTED NITROGEN EXCRETED IN FECES
Esophagojejunostomy bypassing the duodenum	51.5
Roux Y, bypassing the duodenum	44.9
Jejunal segment transplant between esophagus and duodenum	35.7
Colon transplant between esophagus and duodenum	34.8
Esophagoduodenostomy	27.4
Jejunal pouch transplant maintaining esophagoduodenal continuity	25.0

These findings have been further emphasized by the results of the following investigation in which a study in animals with esophagojejunostomy was first made followed by a similar study in the same animals after esophagoduodenal continuity had been re established through a jejunal pouch

METHOD

Total gastrectomy was performed on 2 mongrel dogs using intravenous sodium pentobarbital anesthesia and aseptic surgical conditions Alimentary continuity was initially re established by an esophagojejunal anastomosis with a wide enteroenterostomy which remained in continuity as an esophagojejunostomy bypassing the inverted duodenal stump (Fig 1a) During

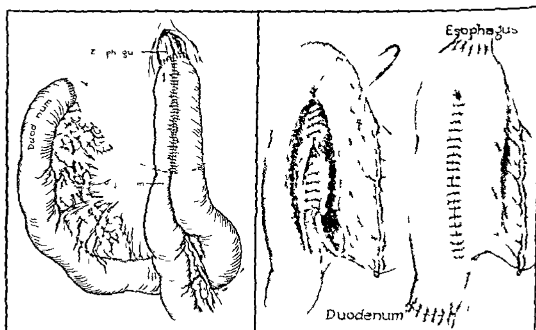


Fig 1 (a) Diagram showing initial reconstruction by esophagojejunostomy following total gastrectomy bypassing the duodenum (b) Diagram showing method of re establishing esophagoduodenal continuity through a revised and transposed jejunal pouch at the second operation

the succeeding 20 months metabolic studies were performed at frequent intervals After this initial period of observation a second operation was performed in each dog and a pouch was fashioned from the proximal jejunum and anastomosed between the esophagus and duodenum to serve as a substitute gastric reservoir (Fig 1b) Following this second procedure metabolic studies similar to those done during the initial period of observation were performed Radiologic films and fluoroscopic examinations were also obtained to visualize the appearance and function of the jejunal pouch

RESULTS

Both animals lost weight following total gastrectomy and did not regain their preoperative weight during the initial period of observation Following modification and transposition of the jejunal pouch at which time it was anastomosed to the previously bypassed duodenum both dogs promptly gained weight to a level above their original preoperative weight (Fig 2) There were no changes in food or dietary habits during these periods of observation

Roentgen studies of the jejunal pouch visualized a substitute gastric reservoir that resembled somewhat the radiologic appearance of a stomach X ray examinations obtained up to 25 minutes after ingestion of barium demonstrated retention of the contrast medium in the jejunal pouch (Fig 3)

Nitrogen excretion studies revealed that 51 per cent of the ingested nitrogen was recovered as fecal nitrogen in the first observation period After alteration and transposition of the jejunal pouch and anastomosing it to the duodenum the fecal nitrogen excretion was reduced to 25 per cent of the total nitrogen intake

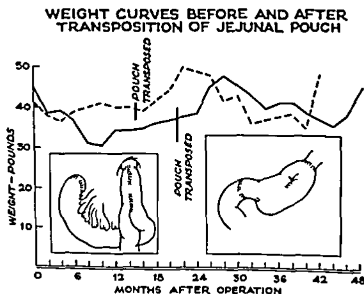


Fig 2 A record of the weights of 2 totally gastrectomized dogs before and after transposition of jejunal pouches showing the effect of bypassing the duodenum The second decline in weight occurred on regular rations The jejunal pouches made it possible for totally gastrectomized dogs to gain weight by taking larger amounts of food immediately after transposition of the jejunal pouch and through the 40th and 48th months respectively

onstrated less fecal nitrogen excretion in those animals in which the duodenum was not bypassed as follows

RECONSTRUCTIVE PROCEDURE	PERCENTAGE OF INGESTED NITROGEN EXCRETED IN FECES
Esophagojejunostomy bypassing the duodenum	51 5
Roux Y bypassing the duodenum	44 9
Jejunal segment transplant between esophagus and duodenum	35 7
Colon transplant between esophagus and duodenum	34 8
Esophagoduodenostomy	27 4
Jejunal pouch transplant maintaining esophagoduodenal continuity	25 0

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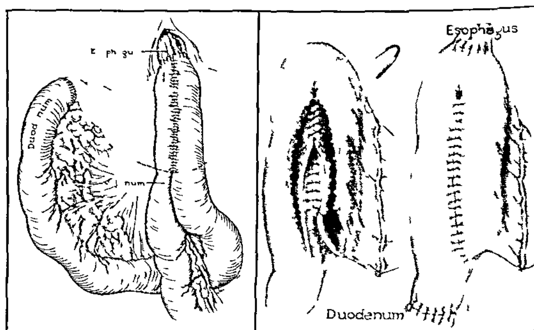


Fig 1 (a) Diagram showing initial reconstruction by esophagojejunostomy following total gastrectomy bypassing the duodenum (b) Diagram showing method of re establishing esophagoduodenal continuity through a revised and transposed jejunal pouch at the second operation

SUPPLEMENTAL EXPERIMENTS IN SYNTHETIC ESOPHAGEAL REPLACEMENT

Edgar F. Berman

From the technical standpoint surgery of the esophagus for the treatment of disease in which there is a demand for replacement of tissue deficits has been fraught with dangerous complications. These results precipitated our attempt some years ago to evolve a technique in which the continuity of the esophagus was resumed by substituting a tissue-compatible plastic tube of the same calibre as normal esophagus. The substitution of the polyethylene tube was a possible key to the solution of this enigma. This work though a practical innovation and still serving a definite need may in retrospect not be considered a finished product.[1,2,3] Experience has shown that there are two disadvantages to this tube technique which must be obviated and which prompted our present supplemental research:

(1) The residual morbidity and mortality due to leakage.

(2) The impingement of the permanent stent on pulsatile or moving structures.

Observing the use of fine nylon cloth as a substitute for resected portions of major blood vessels it was noted that the interstices of the synthetic material became filled with fibrin upon which a fibrous tissue stroma was laid. This becomes lined by endothelium.[4] Our present experiments on the esophagus are extrapolated from this work. It was felt from our previous experience with synthetics in esophageal replacements that in

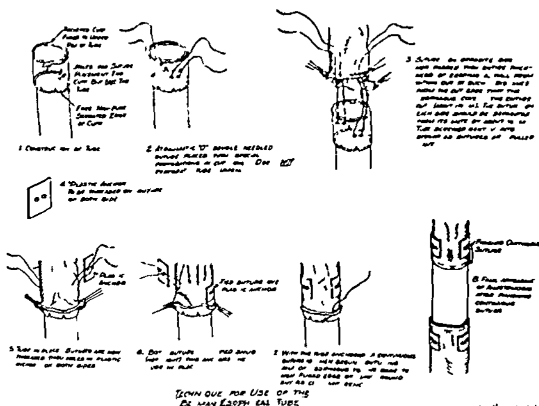


Fig 1



Fig 3 (a) X ray barium contrast film of jejunal pouch used to replace the dog s stomach following total gastrectomy (b) Follow up x ray study taken of the same animal 25 minutes later revealing gradual emptying of contents of the substitute gastric reservoir (jejunal pouch) into the intestine

SUMMARY

1 Total gastrectomy has been performed on 131 dogs and intestinal continuity has been re established by several different surgical techniques Alterations in protein carbohydrate and fat metabolism have been evaluated in these animals The nutrition of animals in which esophagoduodenal continuity was maintained was better than that of animals in which the duodenum was bypassed

2 The nutritional impairment that occurred in 2 dogs following total gastrectomy and esophagojejunostomy bypassing the duodenum was observed for 20 months At a second operation a substitute gastric reservoir was fashioned from jejunum and duodenal continuity was thus restored in both animals Following this second operation improvement in the nutritional status of both of the dogs was observed

REFERENCES

1 Johnson A Goldman P Davis C Harper H McCorkle H J and Gardner R An experimental study of the nutrition of animals following gastrectomy in Surgical Forum 1952 Philadelphia W B Saunders Co 1953 p 40

2 Mineta A K Oppenheimer R O F Harper H A Johnson A H Binkley F M Kerr E E Davis C and McCorkle H J Evaluation of intestinal absorption after total gastrectomy with different methods of re establishment of intestinal continuity in Surgical Forum 1954 Philadelphia W B Saunders Co 1955 p 339

3 Johnson A H Gardner R E Harper H A Binkley F M Bonser Q and McCorkle H J The intestinal absorption of amino acids following gastrectomy in Surgical Forum 1953 Philadelphia W B Saunders Co 1954 p 527

4 McCorkle H J and Harper H A The problem of nutrition following complete gastrectomy Ann Surg *140* 467 1954

5 Johnson A H McCorkle H J and Harper H A The problem of nutrition following total gastrectomy Gastroenterology *28* 360 1955

3 Comment

a The lack of impregnation of the mesh was disappointing in that it was thought that this might be the framework for a new fibrous esophagus with the plastic stent eventually being removed The manner in which the mesh stimulated growth of new tissue was encouraging and its excellent results at the anastamosic ends was interesting

b The infection of the mesh was thought due to the wettability of its fibers with resultant capillary attraction from the normal esophageal ends This results in a mediastinitis It was therefore decided to siliconize the mesh which makes it non wettable (water proof)

Experiment III — Siliconized Elastic Mesh — 10 dogs 1 Same as Experiment II with non wettable (siliconized) elastic nylon mesh sleeves

2 Results

a Same as Experiment II a tight non leaking anastamoses but the mesh does not become infected However it still does not become impregnated and the fibrous tissue grows on the outside of the mesh as before

Experiment IV — Elastic Nylon Mesh Cuffs — 10 dogs 1 a From the evidence gathered in Experiments I II and III it was found that the elastic mesh when between 2 layers of viable tissue (as at the ends of the esophagus) becomes infiltrated by fibrous tissue forming a very firm union This clasps the anastamosis snugly and prevents early leaks by keeping the esophagus close to the plastic tube

b The method used here was the implantation of the plastic tube as before Sleeves of nylon were not used so that a cloth would not be left free within the sheath (especially if the stent was to be removed) Cuffs of nylon mesh were used only over the esophageal ends at the anastamotic areas (The body of the plastic tube was naked no sleeve)

2 Results

a The mesh cuffs at the anastamoses were infiltrated by fibrous tissue A firm union was obvious

b *There were no leaks A fibrous tissue sheath around the naked* plastic tube does not seem to form as fast nor as thick as when the nylon mesh sleeve is used

CONCLUSION

1 The creation of a synthetic substitute for an esophageal deficit by nylon cloth or mesh was unsuccessful

2 The use of elastic nylon mesh cuffs over the anastamotic ends in the plastic tube technique for esophageal replacement prevents postoperative leakage

REFERENCES

1 Berman F F The experimental replacement of portions of the esophagus by a plastic tube Ann Surg *135* 388 1952

2 Berman F F A plastic prosthesis for resected esophagus Arch Surg *65* 916 1952

3 Berman F F Carcinoma of the esophagus a new concept in therapy Surgery *35* 822 1954

4 Deterling R A Jr Shivaj Bhonslay B An evaluation of synthetic materials and fabrics suitable for blood vessel replacement Surgery *38* 71 90 1955

contrast to blood vessel surgery a stent of some kind was necessary (even if temporary), because (1) the nylon cloth is permeable to infected saliva and (2) the cloth may have wrinkled or collapsed prior to fibrous tissue invasion

Experiment I — Nylon Cloth — 5 dogs 1a 4 to 6 cm of midthoracic esophagus was resected

b A plastic tube was substituted by the current technique (Fig 1)

c A nylon cloth sleeve was placed around the tube and tacked to the esophagus at either end

2 Results

a The cloth precipitates an excellent fibrous tissue reaction around the outside of the cloth but not on the inside of the sleeve next to the polyethylene tube

b The nylon cloth was not impregnated by fibrous tissue within 8 weeks except when in apposition to the esophageal ends

c The fibrous tissue provoked by the nylon forms quicker and is heavier than that produced by polyethylene An epithelial layer forms within newly formed fibrous sheath *

Experiment II — Elastic Nylon Mesh Sleeve — 10 dogs 1 Coarse elastic nylon mesh was then used in a manner similar to Experiment I Elasticity allows the nylon sleeve to grip the esophageal ends snugly without obtunding the circulation It was thought that fibrous tissue would impregnate the larger interstices of this mesh (Fig 2)

2 Results

a Excellent results at the anastamoses no leaks Nylon mesh (as previously noted) on the normal esophageal ends becomes pervaded by fibrous tissue and becomes densely adherent to the esophagus A tight anastomoses results

b On the portion next to the plastic tube no impregnation of the interstices of the elastic mesh was found at 8 weeks An extremely quick thick fibrous sheath forms on the exterior of the mesh Epithelium gradually lines the fibrous tissue sheath outside of the mesh

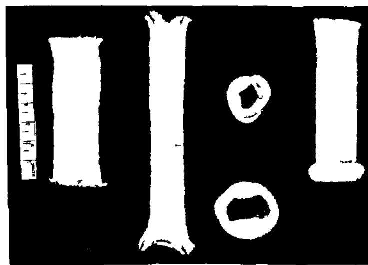


Fig 2 This shows the coarse elastic nylon mesh sleeves and cuffs noting the elasticity of both and the method by which it is used over the plastic tubes

*The elastic nylon mesh tubing was fabricated and donated by The Pyramid Manufacturing Company of New York

medium bevel needles 3 to 4 inches long and varying from #11 to #20 gauge were used. The segment of bowel to be aspirated was grasped *gently* with a moistened sponge and the needle inserted bevel down beneath the serosa on the antimesenteric border. A fairly definite cleavage plane was apparent at the subserosal muscularis junction and the needle was gently forced distally for a distance of 1.5 to 2 cm. At this point a slight downward force thrust the needle through the muscularis into the submucosa where it was again forced distalward for a distance of 0.5 cm before entering the lumen. Aspiration of the distending air was accomplished by suction applied through the ordinary operating room rubber suction tube fitted loosely over the square head of the needle or connected more tightly together through an adapter. *Upon removal* of the needle the site of puncture was pressed firmly between the thumb and forefinger in apposition.

In several of the animals 50 cc of a deeply colored aqueous methylene blue solution was introduced with the air at the first needle puncture in order to make leakage more apparent at subsequent punctures.

It was noted early in the experiment that in the artificially distended animals a total of only 3 punctures were necessary to empty completely the air contained in the small bowel. By very gentle stripping of the intestine between middle and index fingers the entire bowel could be emptied at one puncture. The dog's large intestine was likewise easily emptied at one puncture. For both large and small bowel several reinflations were used to obtain a satisfactory number of deflating punctures.

The obstructed animals reoperated upon after a 24 to 72 hour interval exhibited varying degrees of distention. In all cases of small bowel obstruction the intestine for a variable distance up to 60 cm proximal to the obstruction was distended with gas and fluid discolored and atonic. The large bowel obstructions also exhibited varying degrees of distention but with the expected larger proportion of the distention being due to gas. In these obstructed animals water manometer readings of intraluminal pressure gave no recording above 8 mm. The technique of aspiration was the same as that used previously on the artificially distended animals. During the course of the experiment a total of 276 needle punctures were performed or an average of 11 per dog.

Table 1 Summary of Results

	DISTENTION	NO ANIMALS	PUNCTURES	MORTALITY	AUTOPSY
Small Bowel	Artificial	9	111	1 (26 days) Distemper #3	No evid peritonitis (3)
	Obstruction	6	30	1 (6 days) Distemper #8	Local infl ligature site (1)
Large Bowel	Artificial	8	124	0	Adhesion of omentum to serosal tears (2)
	Obstruction	2	11	0	No evid peritonitis (1)
	Total	25	276	2	7

ASEPTIC DECOMPRESSION OF EXPERIMENTALLY PRODUCED INTESTINAL DISTENTION*

ROBERT C LIEN AND WALTER G MADDOCK

Aseptic decompression of distended bowel by a tube at operation was advocated by Monks[1] as long ago as 1905 Since that time several surgeons but notably Moynihan and Wangensteen[3] have urged the use of various types of aseptic enterostomies to relieve distention at operation The latter s method while of great value has only recently been extensively followed largely because of unwarranted fear of spilling intestinal contents with subsequent peritonitis Much of this concern dates back to Murphy[4] who writing in Ochsner s Surgical Diagnosis and Treatment echoed the sentiments of that day when he stated that the insertion of a tube into the coils of the intestine for withdrawal of contents as advocated by some was a most dangerous practice

It has always been the contention of the senior author that decompression of any distended loops at operation is an essential part of the surgical procedure[5] and we agree with the main thesis of Lord Moynihan[2] who stated that no operation for acute obstruction can be considered complete which leaves an intestine overdistended by contents of an offensive and poisonous nature Without the latter thought and more succinctly stated we believe the abdomen should never be closed over distended intestine

Decompression of distended bowel at surgery employing needles inserted in a tangential fashion has been used by one of us (WGM) and others[6] on numerous occasions without discernible complications However it was felt that an investigation employing needle aspiration of the intestines in a series of dogs would aid in dispelling the fear of peritoneal soilage

METHOD

Twenty five mongrel dogs with an average weight of 10 9 kg were selected for the study Anesthesia was accomplished with intravenous nembutal 30 mg /kg weight In 8 of the dogs a nonstrangulating ligature was applied to the terminal ileum or rectosigmoid and distention allowed to develop spontaneously These obstructed dogs were reoperated upon after intervals of 24 to 72 hours In 17 dogs rubber shod clamps were applied to the duodenum and terminal ileum in the small bowel group and to the terminal ileum and rectosigmoid in the large bowel group and through a needle puncture air was introduced into the intestines to a pressure of 30 to 45 mm of water This tension resulted in a fully distended bowel which after being allowed to remain for 15 to 30 minutes gave the loops a dusky ischemic appearance After the needle aspirations were performed as described later the abdominal wounds were closed and the animals returned to their cages The dogs were followed closely during the postoperative period for clinical evidence of peritonitis No antibiotics were used pre or postoperatively No closure of the needle puncture wounds was done

In order to prevent spillage from the distended bowel the technique of needle aspiration was considered to be important Ordinary hollow bore

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accomplish deflation preoperatively by intranasal short or long tube suction But when this fails decompression of the bowel should be done along with relief of the obstruction at surgery The abdomen should never be closed over distended intestines The patient should be returned from the operating room with the obstructing lesion corrected the intestines flat and a short intranasal tube in the stomach for continuous suction With proper attention to the functioning of this tube, postoperative distention will not occur

SUMMARY

1 A study on 25 dogs is presented, confirming the clinical impression that needle aspiration of gaseous distention at operation properly done is an innocuous and very helpful technique

2 A total of 276 needle aspirations were made in the intestines of dogs distended artificially or obstructed Both clinical examination and autopsies on sacrificed animals revealed no instance of peritonitis The selfsealing nature of the needle tracts was evident A distended bowel containing a methylene blue solution and punctured for decompression showed no leakage when redistended

REFERENCES

1 Monks G H Studies on the surgical anatomy of the small intestine and its mesentery Ann Surg *42* 543 1905
2 Moynihan B Abdominal Operations Philadelphia W B Saunders Co 1926 Ed 4 Vol I 489
3 Wangensteen O H New operative techniques in the management of bowel obstruction Surg Gyn Obst *75* 675 1942
4 Murphy F T Ochsner's Surgical Diagnosis and Treatment Philadelphia Lea & Febiger 2 829 1920 22
5 Maddock W G The importance of air in gastrointestinal distention Surg Clin N America *32* 71 1952
6 Lowdon A G R Deflation of distended bowel at operation Lancet Lond *1* 1103 1951
7 Ochsner A and Storck A H Mechanical decompression of intestine in treatment of ileus effect of stripping on blood pressure Arch Surg *33* 661 1936
8 Morton J J Treatment of ileus Ann Surg *95* 856 1932

RESULTS

Table 1 summarizes the results obtained. It is to be noted that 2 of the dogs died, Number 3 on the twenty sixth and Number 8 on the sixth post operative day. Autopsy of both these animals revealed an acute nasotracheo-bronchitis and bronchopneumonia. They both died within a week of each other during a severe general outbreak of distemper. Dog Number 8 showed localized inflammation about the site of the previously obstructing ileal ligature, but neither dog showed any evidence of leakage at the puncture sites. Dogs Number 1, 6, 19, 22 and 23 were sacrificed at intervals of 14, 9, 1, 7 and 5 days respectively, and searched minutely for evidence of leakage of the aspiration sites. The only consistent finding in these animals was a minute hematoma present in varying stages of resolution at the site of the punctures. Occasionally, where the serosa had been inadvertently split for a distance up to 0.5 cm during the performance of the aspiration, fresh omental adhesions were noted. However, no suppuration or active inflammation was present.

In the several animals tested for leakage by the instillation of methylene blue, the dye traversed to all levels of the gut, but no leakage at deflation puncture sites was seen, even when the bowel wall was stretched again by reinflation.

DISCUSSION

During the course of the experiment a number of factors of importance were noted. Sharp fresh needles were essential. The ease of bowel penetration was directly proportional to their sharpness, which also reduced serosal tearing and muscularis fragmentation. For best results the needles were started with the bevel adjacent to the bowel wall. This assured a minimum of serosal splitting and tended to keep the needle from angling too sharply into the lumen.

The dog's large bowel rather than small intestine was easier to aspirate of its contained gas, due mainly to its increased size and thickness. In the dog the outer longitudinal muscularis is dispersed as a continuous layer and not as taeniae as in the human, and therefore in its human application it is best to aspirate through the taenial bands. In the presence of marked distention such as is found in closed loop colonic obstructions, it is wise to first aspirate with a fine needle to reduce serosal splitting to a minimum.

The hazards of bowel stripping have been enumerated by others[7, 8] but we found it to be a definite and distinct aid in performing the aspirations and have noted no ill effects here or in patients. We wish to emphasize, however, that it should be done very, very gently, and if much fluid is present, simply lifting the bowel will allow the fluid to run downhill to the aspiration point. Punctures, if possible, should be made in areas of relatively normal bowel.

The use of needles, even the relatively large bore #14 gauge, was unsatisfactory for the aspiration of fluid bowel contents. Specially made needles with multiple openings and obturators were tried in an effort to remove fluid but were unsuccessful also. Accordingly, when fluid makes up a considerable portion of the distending material, one of the various trochar procedures best described by Wangensteen should be used.

Readiness to use needle aspiration of distending gas from the intestine during operation in no way lessens the demand for a reasonable effort to

A plastic tube inserted in the femoral vein simplified intravenous therapy after the second operation. Following surgery the dogs were maintained on intravenous fluids for 24 to 48 hours, after which they were given water and food *ad lib*. Intake-output studies and specific gravity studies of blood and peritoneal fluid were conducted as in previous experiments.[2,3,4,5,6,7,8]

RESULTS

Five dogs were subjected to both operations. Three additional dogs with Thiry fistulae were followed to study the bacterial flora of an isolated loop.

During the periods when the Thiry fistulae drained, the dogs remained in excellent health, maintaining their weight and showing no ill effects from the loss of secretions or from the pouring of these secretions over the abdominal wall. All appeared to be in good condition prior to the second operation.

Cultures from the lumen of the isolated loop at varying intervals and at the second operation showed that sterilization of the isolated loop does not always occur (Table 1).

Table 1 Bacteriology

ORGANISM	SOURCE	DOG NO				
		7	17	50	64	93
Clostridium Welchii	Fistula	✓		✓		✓
	P F	✓				
Escherichia coli	Fistula	✓	✓	✓		✓
	P F	✓		✓	✓	✓
Proteus	Fistula	✓	✓			
	P F		✓	✓		✓
Streptococcus	Fistula	✓		✓	✓	✓
	P F	✓	✓	✓	✓	✓
Staphylococcus	Fistula	✓	✓	✓	✓	✓
	P F	✓	✓	✓	✓	✓
Diphtheroids	Fistula				✓	
	P F				✓	✓

The 5 dogs subjected to the second operation had their Thiry fistulae for 64, 97, 126, 291 and 627 days. Following strangulation of the 30 cm segment, the loop changed color and appearance just as in the strangulation obstruction experiments.[3,4,5,6,8] Three dogs survived (Number 17, Number 50, Number 93) and 2 dogs died at 20 and 23½ hours (Number 64, Number 7).

Vomiting occurred twice in 2 dogs and once in 3. None had bloody vomitus. Bloody drainage from the fistula occurred only in the animal that died most rapidly. The maximum temperature deviation was 2.8° above the normal.

The early peritoneal fluid was pink, coagulable, odorless and nonhemolyzed in all experiments. In Number 93 the fluid was purulent, odorless and slightly hemolyzed at 94 hours. In Number 7 the terminal fluid was dark, foul smelling and hemolyzed. This was the only one with *Cl. welchii* in both the peritoneal fluid and the lumen of the fistula.

The autopsy findings on Number 7 were similar to those in strangulation obstruction where no antibiotics were used. The strangulated loop was

STRANGULATION OBSTRUCTION—THIRY FISTULA STUDIES*

A Preliminary Report

ISIDORE COHN, JR.

The Thiry fistula technique has been advocated to gather further information about the toxic substance formed in the bowel in experimental strangulation obstruction.[1] This substance is lethal when absorbed.[8,9] Excluding bile, pancreatic juice, and duodenal secretions from the strangulated segment does not prevent formation of the toxic substance and does not prevent a lethal outcome.[6] Instillation of antibiotics into the strangulated segment prevents formation of this substance and permits the animal to survive even though the strangulated bowel is not resected.[2,3,4,5] This study was part of a continuing attempt to obtain additional information about the nature and mode of formation of this toxic substance as a basis for more rational therapy.

Some advantages of a Thiry fistula for the study of this condition are (1) the better control of the contents of the strangulated segment, (2) the elimination of vomiting and electrolyte loss except as a result of the absorption of toxic materials, and (3) the ability to place specific substances in known concentrations and at a known time directly into the strangulated loop to evaluate their influence on the total picture.

METHOD

Dogs were operated upon under intravenous nembutal† anesthesia and with aseptic technique. Through a midline incision the omentum was removed to prevent its causing too many adhesions and to prevent its providing a blood supply to the strangulated loop following the second operation. The small bowel was divided 75 per cent of the distance from the ligament of Treitz to the cecum and again divided 60 cm. proximal to this point. Proximal and distal bowel were anastomosed end-to-end with a single layer of continuous silk suture. The mesentery of the 2 segments was approximated to prevent herniation. The proximal end of the isolated Thiry fistula was closed with a Parker-Kerr suture. The distal end of the Thiry fistula was exteriorized through a stab wound. The animals were observed for periods varying between 2 and 20½ months. This long time interval allowed the Thiry fistulae to evacuate any biliary, pancreatic, gastric or duodenal secretions which happened to be present at the first operation.

At the second operation, the 30 cm. central segment of the Thiry fistula was completely deprived of its venous supply by isolating, dividing and ligating all of its veins. Both arteries and veins parallel to the bowel at each end of the segment were divided and ligated. This gave a 30 cm. isolated strangulated open loop of small bowel.

Rubber tubes placed in the peritoneal cavity and brought out through stab wounds allowed peritoneal fluid to be aspirated during the survival period.

*From the Department of Surgery, School of Medicine, Louisiana State University, New Orleans, Louisiana. Aided by Research Grant #E-524 (C) from the National Microbiological Institute of the National Institutes of Health, Public Health Service, with acknowledgment to Abbott Laboratories for materials furnished.

†Provided by Abbott Laboratories.

5 Under the circumstances of these experiments life of the animal and viability of a strangulated segment of bowel can be maintained with no support other than adequate blood and fluid replacement

REFERENCES

1 Cohn I Jr A new technic for the study of strangulation obstruction Am Surgeon *20* 563 1954
2 Cohn I Jr Strangulation obstruction—antibiotic protection study A preliminary report in Surgical Forum 1953 Philadelphia W B Saunders Co 1954 p 576
3 Cohn I Jr Strangulation obstruction Postoperative antibiotic protection in Surgical Forum 1954 Philadelphia W B Saunders Co 1955 p 333
4 Cohn I Jr Strangulation obstruction—antibiotic protection Surgery (In press)
5 Cohn I Jr Strangulation obstruction—postoperative antibiotic protection Ann Surg (In press)
6 Cohn I Jr., Celli A Hawthorne H R and Drabkin D L Strangulation obstruction A preliminary report on the effect of diverting duodenal contents in Surgical Forum 1952 Philadelphia W B Saunders Co 1953 p 105
7 Cohn I Jr and Hawthorne H R The role of *Clostridium welchii* in strangulation obstruction Ann Surg *134* 999 1951
8 Nemir P Jr., Hawthorne H R Cohn I Jr and Drabkin D L Cause of death in strangulation obstruction Experimental study I Clinical course chemical bacteriologic and spectrophotometric studies Ann Surg *130* 857 1949
9 Nemir P., Jr Hawthorne H R Cohn I Jr and Drabkin D L Cause of death in strangulation obstruction Experimental study II Lethal action of peritoneal fluid Ann Surg *130* 874 1949

COMPARATIVE STUDIES OF WATER ABSORPTION IN THE DISTAL SMALL INTESTINE AND COLON*

John F Perry Jr.

It was observed in this clinic that patients who had undergone total colectomy and ileoproctostomy for diseases other than ulcerative colitis retained relatively normal bowel function in the late postoperative period if the terminal ileum had been preserved at the time of operation However loss of 30 cm or more of distal ileum with the colon resulted in uniformly poorer results because of persistent postoperative diarrhea [1] This difference suggested that the terminal ileum has a more significant role in the absorption of water than has been credited to it

The purpose of this paper is to report comparative studies of the water absorption capacity of the distal small bowel and colon Our experiments seem to confirm the importance of the ileum in this function at least for the species studied

METHOD

Adult cats of both sexes were used Pentobarbital anesthesia was employed Two segments of bowel were studied simultaneously in each animal and a single experiment was performed on each cat The bowel segment was lavaged with isotonic saline to remove all intestinal content It was then obstructed by ligation at each end with preservation of the blood

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black hemorrhagic, and thick Hemorrhage and edema extended up the mesentery to the point of ligation of the veins The mucosa was black and necrotic The loop contained foul smelling black bloody fluid In Number 64 the peritoneum was filled with bloody fluid and it was thought that death was due to blood loss

Re exploration of the animals that survived revealed the previously strangulated loop had returned to approximately normal color and thick ness The mucosa was normal

Histologic sections of the strangulated bowel from animals that died showed extensive destruction of the bowel wall There was hemorrhage in all layers of the bowel wall in one and a heavy infiltration of bacteria in the other Histologic sections from the animals that survived revealed essentially normal bowel with only slight hemorrhage in the muscularis This resem bled the results obtained in animals protected with antibiotics[2,3,4,5]

DISCUSSION

These preliminary studies have confirmed the usefulness of the Thiry fistula technique for the study of specialized phases of strangulation obstruction

The ability of the animal to eat following strangulation the decrease in vomiting, and the absence of hematemesis made survival studies easier since they simplified postoperative care in general and fluid balance studies in particular

Auto sterilization of the fistulous tract did not occur as had been antici pated but a quantitative decrease in bacterial flora was indicated by the appearance and odor of the drainage from the fistula

The death of one animal was attributed to shock The death of one other animal indicates that death can occur in strangulation obstruction in the absence of shock and without food food products duodenal biliary or pancreatic secretions in the strangulated segment Only those substances produced by or in the strangulated segment are essential for a lethal result Survival of other animals shows that under these circumstances viability of the strangulated bowel and life of the animal can be maintained with no support other than adequate fluid and blood replacement

The possible connection between the presence of *Cl welchii* in both the Thiry fistula and the peritoneal fluid of the animal whose death was not attributed to shock and the absence of this organism from the peritoneal fluid of other animals must not be overlooked[7] Further studies with known quantities of *Cl welchii* placed in the lumen of the Thiry fistula are under way

SUMMARY

1 The successful application of the Thiry fistula technique to the study of strangulation obstruction has been outlined

2 Some advantages of this method of studying the problem of strangula tion obstruction have been discussed

3 Animals which have survived a prolonged period with a Thiry fistula may be subjected to venous strangulation of the fistula without significant untoward results

4 Animals which still harbor a profuse bacterial flora in the Thiry fistula may die when the fistula is strangulated

Table 3. Absorption of Water by Ileal Loops
(Initial Volume 50 cc Isotonic NaCl)

NUMBER OF EXPERIMENT	PERIOD OF ABSORPTION	VOLUME ABSORBED		MIDDLE/TERMINAL
		MIDDLE ILEUM	TERMINAL ILEUM	
1	1 hr	2.5 cc	3.5 cc	.71
2	1 hr	1.0 cc	1.0 cc	1.00
3	1 hr	5.0 cc	10.0 cc	.50
4	2 hr	4.5 cc	6.5 cc	.69
5	2 hr	6.0 cc	5.5 cc	1.09
6	2 hr	8.0 cc	9.0 cc	.89
7	2 hr	11.0 cc	11.0 cc	1.00
8	2 hr	12.0 cc	12.0 cc	1.00
9	3 hr	13.0 cc	10.0 cc	1.30
10	3 hr	16.0 cc	12.0 cc	1.33
11	4 hr	10.0 cc	12.0 cc	.83
12	4 hr	7.0 cc	10.0 cc	.70
13	4 hr	12.0 cc	38.0 cc	.32
14	4 hr	33.0 cc	43.0 cc	.76
15	4 hr	10.0 cc	19.0 cc	.53
16	5 hr	16.0 cc	31.5 cc	.51
17	6 hr	20.0 cc	20.0 cc	1.00
				Mean .86

Table 4. Absorption of Water by Ileal Loops
(Initial Volume 20 cc Homologous Serum)

NUMBER OF EXPERIMENT	PERIOD OF ABSORPTION	VOLUME ABSORBED		MIDDLE/TERMINAL
		MIDDLE ILEUM	TERMINAL ILEUM	
1	30 min	5.5 cc.	1.5 cc.	3.66
2	40 min	3.0 cc	1.5 cc.	2.00
3	40 min	1.0 cc	.5 cc	4.00
4	40 min	6.0 cc	5.0 cc	1.20
5	30 min	2.5 cc	1.5 cc	1.67
6	40 min	1.5 cc	3.0 cc	.5
				Mean 2.18

of water by the 2 segments due to a relatively greater uptake of water by the colon. Still, the absorptive capacity of the terminal ileum was equal to that of the entire colon (Table 2).

Net water absorption by the middle ileum and the terminal ileum under comparable conditions showed certain differences depending on the type of fluid introduced for absorption. When isotonic saline was utilized, mean absorption was slightly greater in the terminal ileum than in the bowel at the higher level (Table 3). With serum, however, the opposite result was obtained. Middle ileum in the presence of a colloid solution absorbed water at a greater rate than did the terminal ileum (Table 4).

Likewise, in the case of the colon, absorption depended on the solution used. With isotonic saline there was no essential difference in absorption

supply and attached to a catheter reservoir system placed 5 cm above the intestinal level During the absorption period the intestinal loops were in the peritoneal cavity Isotonic saline or pooled homologous serum warmed to 37° C was introduced into the loops Periodic raising and lowering of the reservoir allowed uniform mixing of the fluid in the intestinal loop with that remaining in the reservoir Measurement of residual fluid in the reservoir and intestinal segment at the end of the time period could be carried out with an error of about 2 per cent

The following comparisons of net absorptive capacity were made (1) entire colon (excluding the last 3 cm of intraperitoneal colon) with terminal ileum (12 cm segment) (2) terminal ileum (12 cm) with the middle ileum (12 cm) (3) proximal half of the colon with the distal half of the colon

RESULTS

Table 1 shows the results obtained when absorption by the colon and terminal ileum was compared using isotonic saline The average absorption of fluid by a 12 cm segment of terminal ileum was 1 8 times as great as that by the whole colon With serum there was less difference in the absorption

Table 1 Absorption of Water by Terminal Ileum and Colon
(Initial Volume 50 cc Isotonic Saline)

NUMBER OF EXPERIMENT	PERIOD OF ABSORPTION	VOLUME ABSORBED		TERMINAL ILEUM/COLON
		TERMINAL ILEUM	COLON	
1	1 hr	8 0 cc	5 0 cc	1 60
2	1 hr	11 0 cc	6 0 cc	1 83
3	2 hr	19 0 cc	12 0 cc	1.58
4	2 hr	19 5 cc	13 0 cc	1.50
5	4 hr	21 0 cc	20 0 cc	1 05
6	4 hr	14 0 cc	6 0 cc	2.34
7	5 hr	48 0 cc	10 5 cc	4 80
8	5 hr	5 0 cc	12 0 cc	42
9	6 hr	30 0 cc	25.5 cc	1.20
10	6 hr	21 5 cc	11 0 cc	1 91
				Mean 1 82

Table 2 Absorption of Water by Terminal Ileum and Colon
(Initial Volume 20 cc Homologous Serum)

NUMBER OF EXPERIMENT	PERIOD OF ABSORPTION	VOLUME ABSORBED		TERMINAL ILEUM/COLON
		TERMINAL ILEUM	COLON	
1	40 min	2 0 cc	3 0 cc	66
2	40 min	2 5 cc	2 0 cc	1 25
3	40 min	0 5 cc	1 0 cc	.50
4	40 min	5 0 cc	6 0 cc	84
5	40 min	4 0 cc	4 0 cc	1 00
6	40 min	3 5 cc	3 0 cc	1 17
7	40 min	2 0 cc	1 5 cc	1 33
				Mean 97

with the impression that the principal site of resorption of water from the fecal streams is in the proximal half

SUMMARY

Comparisons of water uptake in the ileum and colon of the cat have been made The results support the contention that the distal small intestine is of major importance in water resorption from the bowel

REFERENCES

1 Lillehei R C and Wangensteen O H Total and subtotal colectomies A clinical evaluation Bull Univ Minnesota Hosp and Minnesota Med Found 25 21 30 1953
2 Reid E W On intestinal absorption especially on the absorption of serum peptone and glucose Philos Tr R Soc London 192 215 297 1900

HOMOLOGOUS AND HETEROLOGOUS FREEZE DRIED FASCIA USED TO REPAIR DIAPHRAGMATIC AND ABDOMINAL WALL DEFECTS*

WILLIAM H SEWELL JR AND DOUGLAS R KOTH

Fascia is the strongest tissue readily available to the surgeon that may be used as a free graft in a reconstructive procedure Its mechanical strength[1] is associated with flexibility but not elasticity in the common sense of the word These grafts are tolerated well by other tissues and avoid the theoretical and practical disadvantages of large non absorbable metallic or plastic foreign bodies in the tissue

Free fascial grafts are used occasionally in surgery usually for hernias of various types The primary deterrents in many cases in which it is considered by the surgeon but not used are the operative time trauma and disfigurement of the procedure for the procurement of the tissue

Preserved homografts overcome this objection The cells of such tissues die shortly after implantation even if viable at the time of grafting but the non cellular components often persist for a year or more and act as a framework for invading fibroblasts from the host A firm permanent repair usually results

Successful experimental repair of ventral hernias in dogs by fascia preserved in saline near 0 C was reported by Davis in 1911[3] Koontz in 1926[4] used dog and also ox fascia preserved in alcohol for the same purpose in dogs with success and in 1927 reported successful early clinical results with ox fascia[5] In general however the over all results were disappointing[6]

Homologous tissue such as artery and bone preserved by the freeze drying technique have been used successfully after storage at room temperature for several years both in experimental animals and in human beings[7,8] This technique offers the advantages of ease of storage stockpiling and shipping

* From the Naval Medical Research Institute National Naval Medical Center Bethesda Maryland The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large

Table 5 Absorption of Water by Colon
(Initial Volume 50 cc Isotonic NaCl)

NUMBER OF EXPERIMENT	PERIOD OF ABSORPTION	VOLUME ABSORBED PROXIMAL ½ OF COLON	DISTAL ½ OF COLON	PROXIMAL/ DISTAL
1	1 hr	3 cc	2 cc.	1.50
2	1 hr	2 cc	4 cc	.50
3	2 hr	4 cc	6 cc	.66
4	2 hr	13 cc	8 cc	1 62
5	2 hr	9 cc	10 cc	90
6	2 hr	25 cc	14 cc	1 78
7	3 hr	7 cc	9 cc	78
8	3 hr	8 cc	10 cc	80
				Mean 1 07

Table 6 Absorption of Water by Colon
(Initial Volume 20 cc Homologous Serum)

NUMBER OF EXPERIMENT	PERIOD OF ABSORPTION	VOLUME ABSORBED PROXIMAL ½ OF COLON	DISTAL ½ OF COLON	PROXIMAL/ DISTAL
1	1 hr	4 0 cc	3.5 cc	1 10
2	1 hr	2.5 cc	2 0 cc	1.25
3	1 hr	2 0 cc	1 0 cc	2 00
4	40 min	2 0 cc	0 5 cc	4 00
5	40 min	3 0 cc	2 0 cc	1.50
				Mean 1 97

by the 2 colic segments (Table 5) The absorption of water from serum however was consistently greater in the proximal than in the distal portion of the large bowel (Table 6)

DISCUSSION OF RESULTS

The 12 cm length of ileum arbitrarily chosen for comparison of water uptake with that of the colon represents about one tenth of the combined length of jejunum and ileum in the cat Yet terminal ileal segments of such a length absorb water at a rate equal to or greater than the whole colon Using autogenous serum Reid found the ileum to absorb a greater volume of water per unit of surface area than did the colon Our results would tend to confirm this finding In addition the above experiments suggest that water resorption is an important function of small bowel at least as high as mid ileal levels since net movement of water out of the bowel there is comparable to that of the lower ileum Also the experiments indicate but do not explain certain differences in behavior of both the ileum and colon to colloid and non colloid solutions Apparently the more proximal loops absorb water against greater osmotic gradients than the terminal ileum Likewise absorption seems to occur more readily in the proximal colon than in the distal colon for similar reasons In the colon, this would be in keeping

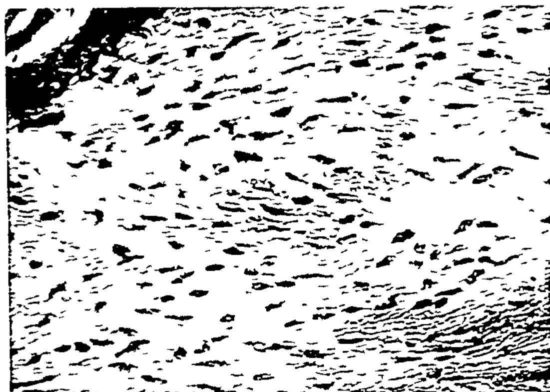


Fig 1 Complete invasion by host fibroblasts into homologous fascia implanted in the abdominal wall of a dog for 8 months H & E stain x210

The series was too small to establish accurately the rate at which the fibroblastic replacement advanced but seemed to be between ½ and 1 mm per month

Microscopic examination of the heterografts revealed much slower and less complete replacement The center of the graft even after 12 months was anuclear This observation signifies persistence of extracellular components of the original graft but failure of the host fibroblasts to penetrate completely because the original nuclei of freeze dried tissue disappear within a few weeks after implantation in an animal

Diaphragmatic Defects Sheets of freeze dried dog and also of calf fascia were *implanted in surgically created defects of the diaphragm* to evaluate the material for possible use in congenital absence of the diaphragm in infants where sufficient autogenous tissue is not available

METHOD

The diaphragm was approached through the bed of the right or the left eighth or ninth rib A rectangular piece of tissue which measured approximately 8 by 10 cm was removed This included a portion of the tendinous region and the majority of the muscular part of the diaphragm on the respective side The defect was repaired with a graft at the same operation again using interrupted 3 0 silk sutures

RESULTS

Homologous fascia was used in 10 of these dogs and heterologous material in 7 The grafts were observed after intervals varying from 1 day to 18 months

Success has been achieved with freeze dried homologous tissues only where the function of the graft is purely mechanical and not metabolic and only with tissues composed primarily of collagen and elastic fibers rather than smooth or striated muscle[9] It is not surprising therefore that Usher *et al*[10,11] were able to report good early results using sutures made of freeze dried human fascia and also ox fascia for repairing hernia in human beings

Animal experiments were designed to study the behavior of freeze dried homologous and heterologous fascia used as sheets for the repair of large defects in dogs, and to investigate some of the basic principles underlying the successful use of this material

METHOD

Ventral Hernias Fascia was taken from the anterior rectus sheath of recently sacrificed healthy dogs or the external oblique aponeurosis of calves slaughtered for meat The grafts were taken without aseptic precautions and were sterilized by exposure to 90 per cent ethylene oxide vapor in air at room temperature and pressure according to a technique previously found to insure complete sterilization[1]

After sterilization the grafts were quick frozen with dry ice alcohol mixture They were dehydrated in a vacuum of 50 microns of mercury pressure for 24 hours and sealed in vacuum in test tubes

A defect was made in one side of the anterior abdominal wall of 15 to 25 kg dogs using aseptic technique A piece of muscle fascia and peritoneum approximately 8 by 10 cm in size was excised In some of the animals the defect was repaired with the graft immediately and the skin and subcutaneous tissue closed over it In others these latter 2 layers were closed and the resulting hernia repaired at a later operation

For the repair the fascial grafts were removed from the ampules and reconstituted in normal saline for 1 or 2 hours They regained their former physical properties and were grossly indistinguishable from fresh fascia They were cut to the size of the defect and implanted with interrupted 3 0 silk sutures

RESULTS

Freeze dried canine fascia was used to repair defects in 7 dogs and bovine grafts were implanted in 6 The dogs were sacrificed after intervals ranging from 1 week to 15 months No wound complications developed in the 9 dogs in which the defect was created and repaired in one stage or in the 4 in which the hernia was repaired at a later operation There were no complete or partial recurrences of the hernias in any of the dogs

At sacrifice the skin was freely moveable over the graft site There were subcutaneous and omental adhesions in every instance These were more prevalent with the heterografts Shrinkage of about 1 cm in both dimensions occurred in most grafts On cut section the heterografts which were followed for the longer periods showed a degenerating center which was not replaced by fibrous tissue.

Microscopic study of the homografts revealed the gradual infiltration of the graft by host fibroblasts which eventually resulted in complete replacement (Fig 1) The replacement from the subcutaneous tissue was more uniform than that from the omental adhesions No foreign body giant cell reaction was found except around the silk sutures Capillary formation was noted

3 Davis J S The transplantation of free flaps of fascia Ann Surg 54 731 748 1911
4 Koontz A R Experimental results in the use of dead fascia grafts for hernia repair Ann Surg 83 [illegible] 1926
5 Koontz A R Dead (preserved) fascia grafts for hernia repair J Am M Ass 89 1230 12[illegible] 192[illegible]
6 Koontz A R Inguinal hernias Am J Surg 82 471 478 19[illegible]
7 Brown R B Hufnagel C A Pate J W and Strong W R Freeze dried arterial homografts clinical application Surg Gyn Obst 9[illegible] 7 [illegible] 19[illegible]
8 Kreuz F P Hyatt G W Turner T C and Bassett C A L The preservation and clinical use of freeze-dried bone J Bone Surg Brit Vol 33A 863 875 195[illegible]
9 Sewell W H Koth D R Pate J W, and Bedell W C Review of some experiments with freeze-dried grafts Am J Surg In press
10 Usher F C Morris C C and Self M M Lyophilized human and ox fascia in the repair of hernias Surgery 36 117 124 1954
11 Usher F C Use of freeze-dried human fascia lata in the repair of incisional hernias Am J Surg 91 [illegible] 19[illegible]
12 Sewell W H Batchelor W H and Koth D R The importance of elastic lamellae in aortic grafts and a technique for the experimental production of aortic aneurysms In Surgical Forum 1954 Philadelphia W B Saunders Co 19[illegible] pp 264 268

Four of the dogs with homografts were explored approximately 1 month after the operation, and all of the grafts were found to be intact All of the homografts in the dogs sacrificed after 4 months or more failed seriously except for 1 There was massive herniation of the abdominal viscera into the thorax covered only by a thin sac

Microscopic study revealed good replacement for a ring of several mm around the edges of the graft but inside of this region the graft was thinned to nearly nothing Replacement from the limited adhesions between the abdominal and thoracic viscera and the graft was insufficient to provide adequate mechanical support

None of the dogs with heterografts had hernias but on cut section the central portion of the grafts again appeared degenerated

DISCUSSION

After implantation freeze dried fascia must be replaced with living fibroblasts in order to maintain its important supportive function The calf fascia used in the present experiment was several times as thick as the homologous material and the results were better in the diaphragmatic site only because some of the original graft substance was still present at the end of the period of observation It appears that complete replacement by host fibroblasts is necessary for permanent success This failed to occur with the heterograft fascia presumably because of a more marked foreign tissue reaction which inhibited replacement Freeze dried heterologous fascia therefore cannot be advised for use in human beings on the basis of these experiments

Homologous fascia is believed to be satisfactory when one entire surface of the graft can be in contact with raw host tissue such as in the repair of various types of hernias but is not advised for the diaphragm On the basis of other work[9] the freeze dried fascia cannot be expected to be successful in an infected wound but it should be absorbed without requiring surgical removal similar to catgut in these circumstances

SUMMARY

1 Pieces of freeze dried homologous and also of bovine fascia were implanted in surgically created ventral hernias of dogs Both were grossly successful after periods up to 15 months but only the homologous fascia was well replaced by host fibroblasts and could have been expected to function satisfactorily for prolonged periods

2 The same graft materials were also placed in defects in the diaphragms of dogs The homografts failed grossly in this site and neither was satisfactory by miscroscopic criteria

3 Sheets of homologous fascia are believed to be satisfactory where one complete surface of the graft is in contact with living host tissue from which replacement can take place but not when the host fibroblasts can grow in only from the edges

REFERENCES

1 Gratz C M Tensile strength and elasticity tests on human fascia lata J Bone Surg Am Vol *13* 334 340 1931

2 Tate J W Sawyer P N Deterling R A Blount J W and Earshley M S Early results in the experimental use of freeze dried arterial grafts In Surgical Forum 1952 Philadelphia W B Saunders Co 1953 pp 147 151

Table 1 Gallbladder Fluorescence Study on Animals

Animal	Number of Animals	Route of Administration	Gallbladder Fluorescence	Time of Exploration or Sacrifice Following Injection	Biliary Duct
Guinea Pig	20	Subcutaneous	++++	24 hours	4+
Dog	15	8 Intravenous 5 Intravenous & subcutaneous 2 Subcutaneous	0 4+	6 28 hours	0-4+
Cats Obstructed	4	Intravenous	0 2+	0 24 hours	0-4+

Table 2 Gallbladder Fluorescence Study on Human Subjects

Patient	Diagnosis	Fluorescence	Dosage	Time (Start Examination)
A	Chronic Cholecystitis	+ Gallbladder — Duct	75 mg	5½ hours
B	Hydrops of Gallbladder	—	1000 mg	13 hours
C	Chronic Cholecystitis Cholelithiasis	—	500 mg	4 hours
D	Acute Cholecystitis	+ Gallbladder + Duct	1000 mg	4 hours
E	Chronic Cholecystitis Cholelithiasis	—	500 mg	51 hours
F	Duodenal Ulcer	Trace in gallbladder	400 mg	2 hours

and time of exploration or sacrifice following injection In only 1 dog was the fluorescent property negative and no explanation is offered for this result

The only case in the obstructed cats which did not reveal some degree of fluorescence of the biliary system following the administration of hematoporphyrin was one which was examined immediately upon the conclusion of the administration In the obstructed animals the thoracic duct revealed a 3+ fluorescence in the animal which was examined immediately upon conclusion of the administration In the remainder the thoracic duct did not fluoresce The mesenteric nodes in obstructed animals exhibited 2 to 4+ red fluorescence in all animals Small bowel content and stool fluoresced from 3 to 4+ in the cases examined Although there was red fluorescent material in the walls of the biliary system in the 3 obstructed animals examined which were sacrificed 24 hours after injection it was interesting to note that the bile in these cases did not fluoresce It was further interesting to note that fluorescence of the common duct appeared only proximal to the point of ligation

Photography* of the fluorescence in color was employed to record the results

* Department of Photography University of California

Clinical and Experimental Problems Relating to Hepatic Coma, The Liver and The Pancreas

FLUORESCENCE OF THE EXTRAHEPATIC BILIARY SYSTEM FOLLOWING PARENTERAL HEMATOPORPHYRIN ADMINISTRATION*

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CHESTER L HERROD GEORGE C PECK H PATTERSON MACK
WILLIAM A HOLBROOK AND FRANK H J FIGGE

It has been demonstrated that porphyrins accumulate in neoplastic embryonic traumatized regenerating, lymphatic, and cancer tissues[1,2,3,4,5,6,7,8] It was observed as an incidental finding that animals which received parenteral hematoporphyrin utilized the liver primarily for excretion Since hematoporphyrin exhibits a typical bright red fluorescence under near ultraviolet light the gallbladder was found to be red fluorescent in autopsied specimens The idea was thus conceived that this property might be of value in clinical gallbladder surgery Subsequently, both animal and clinical studies were undertaken

METHOD

Animal Studies Guinea pigs, dogs and cats were utilized in this experiment The chemical in all cases was hematoporphyrin hydrochloride (recrystallized) The intravenous the subcutaneous and combinations of both routes were employed In the guinea pigs the dosage varied between 50 and 75 mg and in dogs the dosage varied between 100 and 200 mg The time of surgical exploration and/or sacrifice varied between 6 and 28 hours following injection A number of the animals were surgically obstructed prior to the administration of hematoporphyrin (Table I)

Discussion In the case of the guinea pigs the extrahepatic biliary system was noted to fluoresce a brilliant red when exposed to ultraviolet light in all cases In the dogs there were 2 variables i e route of administration

*From the Departments of Neurology and Surgery University of California Medical School and the Department of Surgery Stanford University School of Medicine San Francisco California and the Department of Surgery University Hospital and the Department of Anatomy University of Maryland School of Medicine Baltimore Maryland Supported by grants from the Cancer Research Institute University of California Christine I Webster Research Fund the Committee on Research University of California San Francisco the Anna Fuller Fund the American Cancer Society Maryland Division Inc and the National Institutes of Health Public Health Service (Grants C-4063 and A 73 C)

PANCREATIC AND BILIARY INTRADUCTAL PRESSURES*

RENÉ B. MENGUY, GEORGE A. HALLENBECK, JESSE L. BOLLMAN
AND JOHN H. GRINDLAY

The purpose of this study was to gain a better understanding of the dynamics of the pancreatic and biliary ducts as well as their reciprocal relationships. Such problems as the hypothetical reflux of bile into the pancreatic ducts or the rise in serum amylase after the use of morphine are based on notions of pancreatic and biliary ductal physiology of which, particularly in the case of the pancreatic ducts, our knowledge is far from complete. It was felt that additional information could be gained by studying the pressures within the pancreatic and biliary ducts as well as the variations of these pressures in response to meals and to the administration of various drugs such as analgesic agents and autonomic drugs.

METHOD

Twenty-five dogs were prepared in the following fashion. Polyvinyl tubes (Numbers 21 and 20) were introduced into the pancreatic and common bile ducts respectively, according to the technique described by one of us and his associates.[1,2] These tubes, attached to special introducers, were placed in the ducts so that the open tip of the tube in each main duct was above the sphincter, with the remainder of the tube extending to the surface of the abdomen by way of the parenchyma of the organ, the peritoneal cavity and the abdominal wall. In 10 animals a Number 16 polyvinyl tube was placed in the duodenum for the purpose of recording duodenal motility or injecting various solutions into the lumen of the bowel. In 5 animals cholecystectomy was performed. At least 2 weeks were allowed to elapse between the time of the operation and the actual experiments, during which time the animals were trained to lie quietly on one side. All the experiments were carried out with the animals unanesthetized. Pancreatic and biliary intraductal pressures were measured by connecting the tubes to water manometers. The fluctuations of the columns of water were recorded by means of a photokymograph. The half body thickness at the level of the eleventh rib measured with the animal lying on its side was taken as the manometric zero. The resistance of the pancreatic and biliary sphincters was measured by perfusing the ducts via the indwelling cannulae with normal saline at body temperature and under a constant head of pressure. Control experiments had shown that under normal conditions large amounts of saline similar to those used in the actual experiments could be perfused through the ducts in this manner without any appreciable change in the rate of perfusion. Any significant change in the rate of flow from the control was considered to be due to a change in sphincter resistance.

All the experiments were undertaken with the animals in identical fasting conditions. All drugs were administered intravenously. The action of a given pharmacologic agent was evaluated by comparing the pressures or the sphincter resistance observed during the period subsequent to administration of the drug with those of the period preceding administration.

*From the Mayo Clinic and Mayo Foundation, Rochester, Minnesota, which is part of the Graduate School of the University of Minnesota.

Human Studies Six humans received intravenous hematoporphyrin in dosages ranging from 75 to 1000 mg in time intervals ranging from 1 to 2 hours Exploratory laparotomy was performed within 2 to 51 hours (Table 2)

Discussion Of the 6 humans receiving hematoporphyrin 3 were observed to have a biliary system which was red fluorescent In 1 patient (acute cholecystitis) a 2+ fluorescence of the gallbladder and common duct was observed In this patient an accessory biliary duct was found between the liver and the body of the gallbladder and this duct was found to be fluorescent In 1 patient (chronic cholecystitis) a 2+ fluorescence of the gallbladder and a negative fluorescence of the common duct were observed In the third patient (duodenal ulcer) a trace of fluorescence was noted in the gallbladder only The remaining 3 patients exhibited no fluorescence in the gallbladder or in the common duct

SUMMARY

The utilization of hematoporphyrin as a means of fluorescent visualization of the extrahepatic biliary system was encouraging in the animal studies Continuation of these studies at a clinical level revealed fluorescence of the extrahepatic biliary system in 3 of the 6 patients

REFERENCES

1 Auler H and Banzer G Untersuchungen über die Rolle der Porphyrine bei geschwulstkranken Menschen und Tieren Zschr Krebsforsch *53* 65 68 1942
2 Figge F H J Weiland G S and Manganiello L O J Cancer detection and therapy Affinity of neoplastic embryonic and traumatized tissues for porphyrins and metallo porphyrins Proc Soc Exp Biol N Y *68* 640 641 1948
3 Manganiello L O J and Figge F H J Cancer detection and therapy II Methods of preparation and biological effects of metallo porphyrins Bull School M Univ Maryland *36* 3 7 1951
4 Meyer Betz F Untersuchungen uber die biologische (Photodynamische) Wirkung des Hamatoporphyrins und anderer Derivate des Blut und Gallenfarbstoffs Deut Arch Klin Med *112* 476 503 1913
5 Peck G C Mack H P and Figge F H J Cancer detection and therapy III Affinity of lymphatic tissues for hematoporphyrin Bull School M Univ Maryland *38* 124 127 1953
6 Figge F H J Near ultraviolet rays and fluorescence phenomena as aids to discovery and diagnosis in medicine Bull School M Univ Maryland *26* 165 1942
7 Peck George C Mack H Patterson Holbrook William A and Figge Frank H J Use of hematoporphyrin fluorescence in biliary and cancer surgery Am Surgeon *21* 181 188 1955
8 Rasmussen Taxdal David Ward Grant E and Figge Frank H J Fluorescence of human lymphatic and cancer tissues following high doses of intravenous hematoporphyrin Cancer Phila *8* 8 81 1955

Table 1. Effects of Some Analgesics on Pressures in the Pancreatic Duct of Fasting Dogs

DRUG	DOSE MG./KG.	EXPERIMENTS	PRESSURE, MM. OF WATER: MEAN INCREASE*	RANGE OF INCREASE	MAXIMAL	DURATION OF INCREASE, MIN.
Morphine	0.2	104	101.4	19–205	320	55–100+
Codeine	[illegible]	64	39.5	24–51	242	10–110+
Methadone	0.2	34	84.[illegible]	48–110	2 7	20–120
Dromoran	0.08	24	63.2	48–78	232	75
Levo-dromoran	0.05	14	60		205	8
Demerol	2	64	71.5	36–117	225	35–140

* Mean difference between pressure before the injection and maximal pressure during 2 hours after the injection.

† Pressure increased in all cases.

in the tables. To some degree and for varying periods the pancreatic intraductal pressures were increased by all these agents given in clinical doses. Meperidine hydrochloride (demerol) also had this effect (Table 1). It is obvious from Table 2 that the biliary pressure was affected little if at all by the analgesics and increases in pressure were usually transitory. On the other hand, when the same doses were administered to cholecystectomized animals the biliary intraductal pressure invariably climbed to maximal levels (Table 3).

Perfusion studies after the administration of morphine and demerol showed that these drugs caused a marked increase in the resistance of the pancreatic and biliary sphincter mechanisms.

Three animals which showed a great increase in pancreatic pressure after taking morphine showed a transitory elevation in the serum amylase. In 2 other animals in which the change in pressure was less marked the concentration of enzyme in the serum was not altered.

DISCUSSION

In dogs the relationship between the pancreatic and the biliary intraductal pressures is such that, if the data can be extrapolated to the anatomic conditions existing in the human being, a reflux of bile into the pancreatic duct would be highly improbable even during a prolonged spasm of the sphincter of Oddi. On the other hand, in view of the marked lability of the biliary pressures in the cholecystectomized animals, a reflux of bile might occur in cholecystectomized patients or in patients with nonfunctioning gallbladders.

The increase in the concentration of amylase in the serum after the administration of morphine in some of our experiments was related to stasis in the pancreatic ducts. It is possible that the mechanisms of the action of morphine on the serum amylase in the human being is similar.

Further experimentation with simultaneous perfusion of the ducts and recording of duodenal motility has led us to suspect that the action of morphine on the pancreatic and biliary sphincters is largely correlated to the effects of these agents on the smooth muscle of the duodenum. The period of increased resistance of the sphincter after morphine was admin-

of the drug Separate control experiments showed that intravenous injections of saline did not alter the intraductal pressures or the resistance of the sphincter

Pressures During Fasting In fasting dogs the pancreatic intraductal pressure was always higher than the biliary pressure The mean pressures observed were 139 9 mm of water for the pancreatic duct and 70 9 mm of water for the common bile duct The biliary pressure was higher in the cholecystectomized animals and in this group a mean pressure during fasting of 111 9 mm was observed in the common bile duct In 1 of the cholecystectomized animals it was possible to measure simultaneously the pancreatic and biliary pressures the pressure in the bile duct was found to be consistently higher than that in the pancreatic duct

Meals As previously described by Parry and associates[2] pressures tended to increase simultaneously in both the pancreatic and common bile ducts after a meal The degree of elevation was usually moderate and varied from dog to dog The pancreatic intraductal pressure except for occasional brief periods was usually higher than the biliary pressure In the cholecystectomized animals on the other hand the already high biliary pressure tended to drop after a meal and then to remain relatively stable

Drugs *Autonomic Nervous System Effector Drugs* In most instances pilocarpine in doses of 0 2 mg per kg of body weight produced a moderate increase in both pancreatic and biliary intraductal pressures as well as an increase in the frequency and the amplitude of the pressure waves In the intact dogs the increase in pressure in the common bile duct was frequently very abrupt suggesting a forceful emptying of the gallbladder In the cholecystectomized animals the biliary pressure increased slightly after an injection of pilocarpine but the gradient was never as steep as in the animals having gallbladders The resistance of the pancreatic and biliary sphincters was found to be increased by pilocarpine This increase in resistance was fluctuating in nature with periods when the flow was as rapid as or more rapid than during the controls

Atropine in doses of 0 15 mg per kg of body weight almost invariably produced a prolonged drop in the pancreatic intraductal pressure as well as an appreciable decrease in the resistance of the pancreatic sphincter mechanism The action of atropine on the biliary intraductal pressure was rather inconsistent In the cholecystectomized animals a slight drop in pressure was usually noted after the injection of atropine but when the experiment was pursued long enough a secondary increase in pressure above the control was observed Similarly the effects of atropine on the resistance of the biliary sphincter were often inconsistent and paradoxic

Epinephrine given intravenously at the rate of 1 gamma per kg of body weight per minute invariably produced a marked increase in the resistance of the biliary sphincter mechanism as well as a rise in the biliary intraductal pressure a rise that was more pronounced in the cholecystectomized animal Epinephrine did not alter appreciably the resistance of the pancreatic sphincter however it tended to decrease considerably the flow of pancreatic juice and resulted in a slight decrease in pressure

Ergotoxine ethanesulfonate a sympatholytic agent produced a slight increase in both pancreatic and biliary intraductal pressures

Analgesic Agents The effects of certain analgesic agents are outlined

Table 5 Effects of Some Analgesics on the Pressure in the Biliary Ducts of Cholecystectomized Fasting Dogs

DRUG	DOSE MG /KG	EXPERIMENTS	PRESSURE MM OF WATER: MEAN INCREASE	RANGE OF INCREASE	MAXIMAL	DURATION OF INCREASE MIN
Morphine	0.2	4†	158	137 176.5	308	60 120
Codeine	2	2†	192	169 215	309	100 105+
Methadone	0.2	2†	172	156-187.5	315	60 88
Dromoran	0.08	2†	201	190.5 221.5	320	40 150
Levodromoran	0.05	2†	191.5	183 206	310	90 140
Demerol	2	4†	123.5	85 162	269	30 90

Mean difference between the pressure before the injection and the maximal pressure within 2 hours after the injection

†Pressure increased in all cases

istered seems to correspond more to the secondary phase of the drug s effect on the duodenum that is to the prolonged secondary phase of decreased or absent motility rather than to the initial phase of increased motility and tone

SUMMARY

By means of a method of measuring pancreatic and biliary intraductal pressures that leaves the sphincteric mechanisms of the ducts intact we have studied the response of these pressures to meals and to various drugs In dogs the pancreatic intraductal pressure was always found to be higher than that in the common bile duct After cholecystectomy the biliary pressure was found to be higher than in the intact animals After meals an increase in pressure was observed in both ducts Analgesic agents produced an elevation in pancreatic intraductal pressures without any concomitant rise in the biliary pressure the latter occurring only in the cholecystectomized animals The effects of various autonomic drugs on these pressures have also been studied

REFERENCES

1 Grindlay J H Eberle J and Walters W Technique for external drainage of the biliary tract which leaves ducts intact an experimental study A M A Arch Surg *67*:289 96 1953

2 Parry E W Hallenbeck G A and Grindlay J H Pressures in the pancreatic and common ducts values during fasting after various meals and after sphincterotomy an experimental study A M A Arch Surg *70* 757 65 1955

Table 2 Effects of Some Analgesics on Pressure in the Biliary Ducts of Intact Fasting Dogs

DRUG	DOSE MG/KG	EXPERIMENTS	INCREASE	DROP	NO CHANGE	PRESSURE MM OF WATER: MEAN CHANGE*	PRESSURE MM OF WATER: RANGE OF CHANGE	PRESSURE MM OF WATER: MAXIMAL	DURATION OF INCREASE MIN
Morphine	0.2	5	1	1	3	+6.5	−17 to +30	95	0 30
Codeine	2	4	3	0	1	+43	30 58	119	8 20
Methadone	0.2	2	2	0	0	+58.5	49 68	135	15-43
Dromoran	0 08	2	1 (delayed)	1 (initial)	1	+22.5	−33.5 to +56	146	—
Levodromoran	0 05	2	1	1	0	−10	−35 to +15	91	—
Demerol	2	2	2	0	0	+20.2	20 20.5	74	5 11

*Mean difference between the pressure before the injection and the maximal or minimal pressure within 2 hours after the injection

Table 1. 328 Blood Ammonia Determinations on 48 Patients

	RANGE OF BLOOD AMMONIA NITROGEN IN MICROGRAMS PER CENT
No hepatic dysfunction (20 patients, 76 determinations)	40-86
Hepatic dysfunction, no neurological symptoms (15 patients, 92 determinations)	70-180
Hepatic coma or pre-coma (13 patients, 160 determinations)	50-530
Highest level in 24 hours preceding onset of neurological symptoms	190-440

tients in coma or pre-coma ranged from normal to greatly elevated values there invariably was a significant elevation of blood ammonia preceding the onset of coma. The dynamic relationship between blood ammonia nitrogen level and clinical status will be illustrated by the detailed cases presented below.

Effect of Glutamate on Blood Ammonia Nitrogen Level. The changes in blood ammonia nitrogen concentration accompanying the infusion of 25 gm. of sodium glutamate in 1 hour are shown in Fig. 1. The fall in blood ammonia nitrogen ranged from 80 to 210 μg. per cent; the effect was maximal from 1 to 2 hours after the start of the infusion; the duration of significant effect was at least 4 hours. No deleterious effects were noted.

BLOOD AMMONIA NITROGEN AFTER INFUSION OF 25 GM OF SODIUM GLUTAMATE IN ONE HOUR

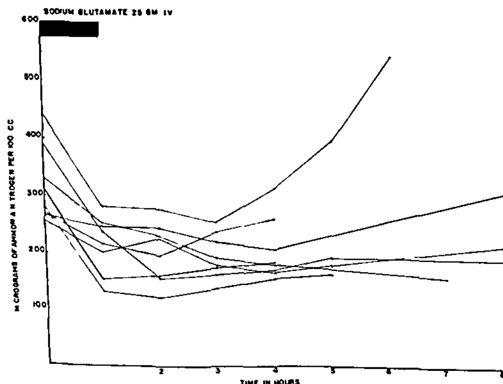


Fig. 1

THE EFFECT OF GLUTAMATE ADMINISTRATION ON BLOOD AMMONIA LEVEL AND ITS CORRELATION WITH CLINICAL STATUS IN PATIENTS WITH HEPATIC COMA*

DONALD M PEARLMAN

Although sodium glutamate has been used sporadically in the treatment of hepatic coma since Walshe[1] reported its successful use in 3 patients there have been no precise data on its efficacy or pharmacological action Walshe suggested that the coma of liver failure was due to an increase in intracellular brain ammonia He administered glutamate which will bind ammonia to form glutamine

The relation between ammonia metabolism and hepatic coma still has not been clarified although it has been studied since 1927[2] McDermott *et al*,[3] however determining blood ammonia levels daily have recently reported a significant but imperfect correlation between blood ammonia level and clinical status

In the study to be reported below blood ammonia determinations were done on a variety of individuals including some in hepatic coma as often as every hour Determinations were carried out before during and after administration of glutamate and the relation of the blood ammonia level to clinical status was determined

METHOD

Multiple blood ammonia determinations were carried out on 20 normal controls 15 patients with clinical and laboratory evidence of hepatic dysfunction and no neurological symptoms and 13 patients who at some time in their hospital course presented the characteristic symptoms of hepatic coma or pre coma

The blood ammonia concentration was determined by a modification of the Conway micro-diffusion technique essentially identical to that described by McDermott and Adams[4] Determinations were made daily on stable patients and as frequently as hourly on patients who showed any change in clinical status or in any known factor which would influence ammonia metabolism

Five patients in hepatic coma received intravenous infusions of 25 gm of sodium glutamate in 1 hour Three of the 5 received 2 infusions each The intravenous infusion was prepared by making up a 10 per cent solution of commercial monosodium glutamate in water and autoclaving it Three of these patients also received glutamic acid 2 gm per hour by nasogastric tube

RESULTS

Normal and Abnormal Blood Ammonia Levels The range of blood ammonia levels in all patients studied is shown in Table 1

It is noteworthy that while the blood ammonia nitrogen levels of pa

*From the Department of Surgery Yale University School of Medicine and the Samuel B Harvey Metabolic Laboratory New Haven Conn Supported by a grant from the Fluid Research Fund Yale University School of Medicine

The author wishes to acknowledge his appreciation to Dr William V McDermott Jr Dr Gerald Klatskin Dr Mark Hayes and Dr William Glenn for their generous assistance

LAENNEC'S CIRRHOSIS WITH CARBON TETRACHLORIDE POISONING

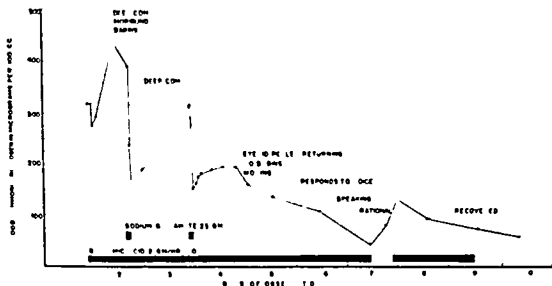


Fig 3

PRIMARY BILIARY CIRRHOSIS WITH BLEEDING ESOPHAGEAL VARICES

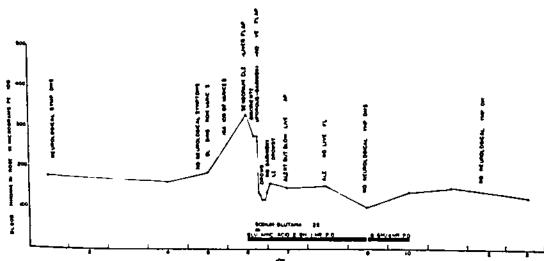


Fig 4

per cent following the second infusion it was not until the blood ammonia nitrogen had been under 200 μg per cent for over a day that there was any decrease in the depth of the coma

Fig 4 illustrates the course of a 47 year old female with primary biliary cirrhosis who entered the hospital for a portacaval shunt because of previous bleeding from esophageal varices While in the hospital she again bled from her varices which were then ligated transesophageally

The sharp rise in blood ammonia concentration followed the accumulation of protein (blood in this case) in her intestine and the insult of her liver of anoxia and anesthesia Coma did not ensue immediately following the rise in blood ammonia concentration but 8 or 10 hours later when the level was already falling In this case which was characterized

from the infusion The only factor limiting the amount of sodium glutamate which could be administered was the alkalosis and hypernatremia produced

Clinical Effects of Changes in the Blood Ammonia Concentration Of the 5 patients treated with glutamate 2 died without apparent decrease in their coma 1 improved somewhat but relapsed into deep coma and died and 2 made full recoveries

Both of the patients who died without change in their coma were far advanced cirrhotics who gradually decompensated went into coma and died Glutamate infusion produced only transient (1 to 6 hour) drops in blood ammonia concentration which rapidly rose to even higher levels post infusion Clinical courses of the 3 patients who demonstrated changes in neurological status during glutamate therapy are charted in Figs 2 3 and 4

Fig 2 illustrates the course of a 56 year old woman with posthepatitic cirrhosis and bleeding esophageal varices On admission a Sengstaken Blakemore tube was inserted and she was given glutamic acid 2 gm per hour through the tube

The patient began to deteriorate however and on the fifth hospital day bled from her stomach below the gastric balloon Here again transient falls in the blood ammonia level were not associated with change in the depth of coma

Fig 3 illustrates the course of a 18 year old female with an alcoholic past who was exposed to carbon *tetrachloride 11 days prior to admission* Icterus and oliguria developed and on the day prior to admission the patient became lethargic

Although the blood ammonia nitrogen fell to 160 μg per cent immediately following the first infusion of sodium glutamate and to 152 μg

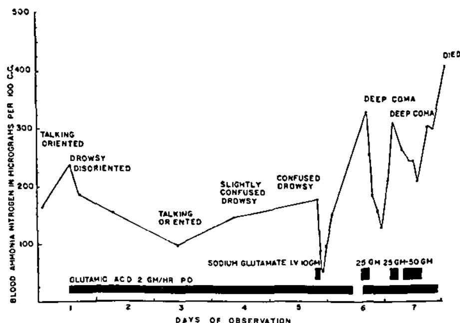


Fig 2

THE ROLE OF AMMONIA IN THE PRODUCTION OF HEPATIC COMA*

BEN EISEMAN, W C FOWLER, PHIL J WHITE AND
GLENN M CLARK

Several clinical and laboratory observations have implicated circulating blood ammonia as the toxic agent which is partially or wholly responsible for hepatic coma[1,2,3,4] None of these observations however has been conclusive The purpose of this paper is to describe an experimental method by which a neurologic state indistinguishable from hepatic coma is produced in dogs by means of ammonium salt infusion into the carotid artery and to describe some of the biochemical lesions found in the brain at the height of such coma

METHOD

Adult mongrel dogs fed routine kennel rations were utilized throughout this study The day prior to the experiment with the animal under light intravenous pentobarbital anesthesia polyethylene tubing was placed in the common carotid artery and the external jugular vein This tubing was sewn to the vessel with arterial silk filled with a heparin saline solution to prevent clotting fixed to the overlying skin and then enclosed in a plaster of Paris collar encircling the neck of the animal These precautions were taken to assure patency and to prevent dislodgement of the tubing from the vessel A cranial bone flap was reflected extending from the sagittal suture medially to the zygomatic process laterally thus exposing the anterior portion of one hemisphere The dura was left intact the bone flap loosely replaced and the soft tissues loosely closed over the defect The animal was then given 100 000 units of penicillin and 0 5 gm of streptomycin Upon recovery from anesthesia the animal was given a routine kennel diet

Twenty four hours after such preparation the unanesthetized dog was placed on his side on an animal operating table the neck cast was removed and the ligated ends of the polyethylene cannulae opened The test solutions were immediately infused into the carotid artery cannula under positive pressure by utilizing a blood pressure bulb attached to a needle entering an ordinary intravenous flask The tube in the jugular vein was utilized for the withdrawal of blood samples and was kept patent with a slow infusion of 5 per cent glucose in water

Solutions used in this study included 5 per cent glucose in water (control) 4 per cent ammonium citrate in 5 per cent glucose in water 5 per cent ammonium chloride in 5 per cent glucose in water 1 per cent ammonium hydroxide in lactate Ringers solution and 1 per cent ammonium hydroxide in 5 per cent glucose in water adjusted to pH 8 3 by the addition of hydrochloric acid

Heparinized blood samples were simultaneously withdrawn from an indwelling cannula in a femoral vein and from the jugular cannula at appropriate intervals during the experiment Ammonia content was deter

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by acute onset of coma and short duration of elevation of blood ammonia recovery followed the fall in blood ammonia level more quickly than in the case illustrated in Fig 3 Of interest is the appearance of the peculiar flapping tremor described by Adams and Foley[5] as indicative of impending hepatic coma at the height of the blood ammonia level its disappearance as coma ensued and its subsequent reappearance and final disappearance upon recovery

DISCUSSION AND SUMMARY

In the cases of hepatic coma studied changes in neurological status occurred only following major changes in blood ammonia level of significant duration Single or even daily determinations may fail to reveal the relationship of blood ammonia level to clinical status The data collected are consistent with the hypothesis that the brain does not come into instantaneous equilibrium with the blood as to ammonia content but rather absorbs ammonia from or discharges ammonia to the blood at a rate so slow that moderate changes in blood ammonia level affect the neurological status of the patient only after many hours The demonstration by Bessman *et al*[6] of a significant difference between arterial and jugular bulb blood ammonia levels in cirrhotics in hepatic coma and no difference between these levels in non comatose cirrhotics tends to support this hypothesis

Significant drops in the blood ammonia level follow the administration of glutamate *All* changes in clinical status *following glutamate infusion* could be explained by its effect on blood ammonia concentration Glutamate apparently is of value in treating hepatic coma in cases in which the liver retains some capacity to recompensate.

REFERENCES

1 Walshe J M The effect of glutamic acid on the coma of hepatic failure Lancet Lond *263* 1075 1951
2 Burchi R Saggio della funzionalita epatica con lo studio della ammoniemia spontanea e provocata Folia clin chim Bologna n s 1 3 35 1926 Abstracted in Kongrzbl inn Med *47* 80 1927
3 McDermott W V Jr Adams R D and Riddell A Ammonia metabolism in man Ann Surg *140* 539 1954
4 McDermott W V Jr and Adams R D Episodic stupor associated with an Eck fistula in man with particular reference to ammonia metabolism J Clin Invest *33* 1 1954
5 Adams R D and Foley J M The neurological disorder associated with liver disease Res Pub Ass Res Nerv Ment Dis *32* 198 1953
6 Bessman S P Fazekas J F and Bessman A N Uptake of ammonia by the brain in hepatic coma Proc Soc Exp Biol N Y *85* 60 1954

control brain specimen biopsies were simultaneously analyzed on animals prepared as were the infused animals but rendered unconscious immediately prior to obtaining the biopsy specimen by intravenous pentobarbital anesthesia (4 dogs) or by local infiltration with 1 per cent procaine (1 dog).

RESULTS

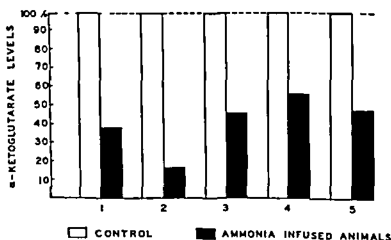
Progressive neurologic abnormalities indistinguishable from advancing hepatic coma were produced in each animal receiving a carotid artery infusion of an ammonium salt solution. After periods varying from 1 to 5 hours the animal began to show convulsive twitching of all extremities and peripheral reflexes became hyperactive. Such hyperactivity gradually was replaced by hypoactivity as the dog slipped into coma. Occasionally during coma there were minor convulsive twitchings. The dog would expire in coma if the ammonium salt infusions were continued. If the rate of infusion were diminished, coma could be prolonged. If the infusion were stopped coma would disappear to reappear shortly after reinstituting ammonium salt administration.

In no case did carotid artery infusion of the ammonium salts produce

Animal Number	α Ketoglutarate Level μg/GM	% Diminution from Control
1 a 531	6.9	
b 542	2.6	38
2 a 531	6.9	
b 551	1.15	17
3 a 605	1.5	
b 405	0.48	32
4 a 586	3.1	
b 610	1.8	58
5 a 665	2.8	
b 655	1.38	49

(a = Control)

a Absolute values



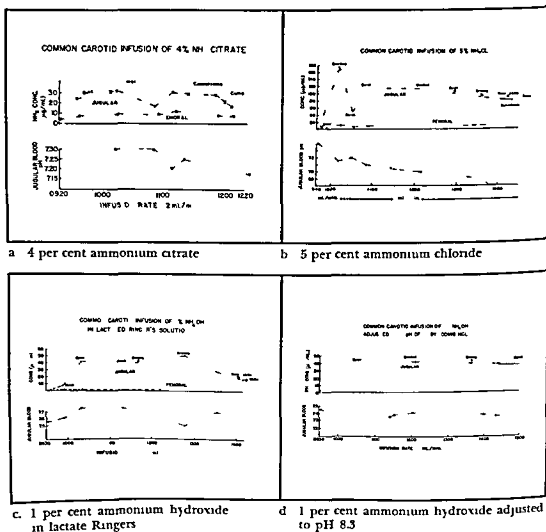
b Graph showing reduction of alpha ketoglutarate compared to paired controls

Fig 2 Brain alpha ketoglutarate concentration of animals in coma due to ammonia intoxication

mined within 5 minutes after withdrawing the blood sample according to the Conway microdiffusion technique[5] Measurements of blood pH were performed on the Beckman potentiometer

Observation and recording of the level of consciousness reflexes respiratory rate and pulse were made during the infusion At the height of coma, or at a similar interval in the controls, the loosely sutured scalp incision was quickly opened the bone flap removed and a wide cruciate incision made in the dura thus exposing the brain An ordinary teaspoon modified slightly to fit within the craniotomy wound was then used to scoop out a generous portion of the frontal parietal and occipital lobes of the exposed hemisphere of the comatose animal This brain specimen which was approximately 5 cm in diameter was immediately dropped into a beaker of liquid nitrogen for instantaneous freezing

Alpha ketoglutarate determinations on the brain samples were done according to a modification of the LePage technique[6] In essence this consisted of extracting alpha ketoglutarate from a frozen brain sample with 2 4 dinitrophenylhydrazine separating it on an absorption column and quantitating the alpha ketoglutarate band spectrophotometrically Paired



a 4 per cent ammonium citrate

b 5 per cent ammonium chloride

c. 1 per cent ammonium hydroxide in lactate Ringers

d 1 per cent ammonium hydroxide adjusted to pH 8.3

Fig 1 Effects of ammonium salt infusion into carotid artery of unanesthetized dogs

This is in keeping with our clinical experience in which blood ammonia elevation does not invariably reflect the clinical status[7]

Previously we as well as others[3, 7, 8] have suspected that the primary biochemical lesion of ammonia intoxication is an interruption in the Krebbs cycle by the combination of ammonia with alpha ketoglutarate in the formation of glutamate (Fig 3) Such a reaction resulting from an excess of ammonia removes an essential substance (alpha ketoglutarate) from the dicarboxylic energy cycle and might produce the clinical abnormalities known to accompany hepatic failure and ammonia intoxication Biochemical evidence for such a concept has heretofore been lacking but the data of these experiments support this hypothesis

CONCLUSIONS

1 Neurologic changes indistinguishable from those of hepatic coma have been produced in unanesthetized dogs by infusion of various ammonium salts into the carotid artery

2 Such changes are independent of alteration in blood pH

3 Prolonged periods of elevated blood ammonia levels must be maintained before coma can be produced

4 The alpha ketoglutarate level of brain tissue has been shown to decrease markedly in animals in coma due to ammonia intoxication The significance of this biochemical abnormality in the Krebbs cycle is briefly discussed

REFERENCES

1 Folin O and Denis W The origin and significance of the ammonia in the portal blood J Biol Chem *11* 161 167 1912

2 Phillips G B Schwartz R Gabuzda G J and Davidson C S The syndrome of impending hepatic coma in patients with cirrhosis of the liver given certain nitrogenous substances N England J M *247* 239 246 1952

3 McDermott W V and Adams R D Episodic stupor associated with an Eck fistula in the human with particular reference to the metabolism of ammonia J Clin Invest *33* 1 9 1954

4 Riddell A G Kopple P N and McDermott W V The etiology of meat intoxication in the Eck fistula dog Surgery *36* 675 684 1954

5 Conway E J Apparatus for the microdetermination of certain volatile substances The blood ammonia with observations on normal human blood Biochem J Lond *29* 2755 2772 Pt 2 1935

6 LePage G A Measurements of ketoacids in normal and neoplastic rat tissue Cancer Res *10* 393 397 1950

7 Eiseman B Bakewell W and Clark G Studies in ammonia metabolism I Ammonia metabolism and glutamate therapy in hepatic coma Am J Med (in press)

8 Bessman S P Fazekas J F and Bessman A N Uptake of ammonia by the brain in hepatic coma Proc Soc Exp Biol N Y *85* 66 67 1954

coma in less than 1½ hours, despite prolonged marked elevation of the jugular vein ammonia levels (Fig 1)

Similar results were obtained with each of the ammonium salt solutions proving that the reaction was characteristic of the ammonium radicle and not caused by citrate or other anion toxicity

Both ammonium chloride and ammonium citrate administration produced varying degrees of acidosis Mild alkalosis or no change in acidity resulted from the administration of ammonium hydroxide buffered in lactate Ringers solution It is, therefore evident that the neurologic changes were not dependent upon alteration of the pH of the blood

Administration of 5 per cent glucose in water for similar periods and at the same rate as the ammonium solutions did not produce neurologic changes This control experiment was performed in order to exclude the possible effect of water intoxication in producing neurologic abnormalities

In each case brain alpha ketoglutarate concentration of the comatose animals was markedly diminished compared with its paired control (Fig 2) Although there was variation of these values in different runs even on normal brain samples, our experimental method was consistent when paired samples were utilized It is evident that the chemical method of alpha ketoglutarate determination though consistent within a single run cannot serially produce results of repeatable absolute values The significant fact remains that ammonium salt administration to the point of coma resulted in marked diminution of the brain alpha ketoglutarate concentration

DISCUSSION

In these experiments the neurologic changes of ammonia intoxication appeared only after prolonged elevations of blood ammonia concentration

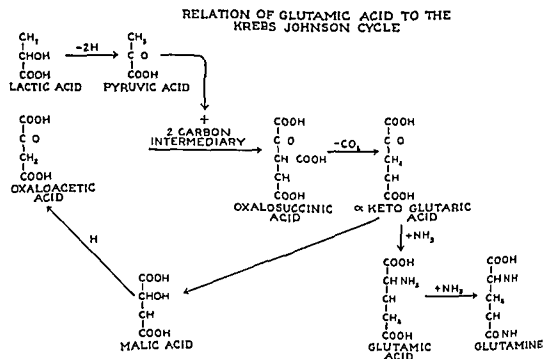


Fig 3 Krebbs cycle showing relation of ammonia to alpha ketoglutarate

amputated this structure was likely to become attached to the various margins of the segment of intestine in such a way as to nullify the intended exposure of the mucosa

Twenty two dogs were used in the definitive experiments In each of the animals ascites was produced according to the method of Schilling McKee and coworkers[1,2] In brief at thoracotomy, a metal band of 1/4 inch width was placed about the inferior vena cava above the diaphragm and tightened to constrict the vessel to approximately one half its original diameter Such constriction was sufficient to lead to the development of ascites within 2 weeks in all animals but one The dogs were maintained on a standard kennel ration which provided approximately 2 gm of salt daily

Of the 22 animals so prepared 8 died immediately after operation of miscellaneous acute causes leaving 14 animals for protracted study These were divided into 2 groups One group of 6 animals was set aside as controls to permit observation as to the persistence of ascites developing after constriction of the inferior vena cava The second group of 8 animals was subjected to a second operative procedure approximately 2 to 3 weeks after the caval constriction This procedure was preceded by the administration of aureomycin and neomycin to reduce the intestinal bacterial flora and consisted of the removal, with blood supply intact from the course of the ileum of a segment of intestine 1 to 8 inches in length After ileoileostomy to restore the continuity of the intestinal tract the specified segment was incised through the full thickness of its wall along the antimesenteric border leading to the development of a flattened sheet of tissue which was then sutured to the peritoneum of the anterior abdominal wall in such a way as to expose the mucosa to the peritoneal cavity Before closure of the abdominal wound the omentum was amputated

RESULTS

Ascites developed within 2 weeks in all of the 6 control animals subjected to constriction of the inferior vena cava and persisted uninterruptedly from its onset These animals have been observed for periods of time up to 6 months and in none has spontaneous regression of ascites occurred The animals have required paracenteses at intervals of 3 weeks and have yielded 2000 to 3000 cc of ascitic fluid at each tap

In the 8 animals subjected to exposure of ileal mucosa within the peritoneal cavity after constriction of the inferior vena cava the collection of ascitic fluid either did not recur or recurred in greatly reduced quantities One of the 8 animals required 1 paracentesis this animal was then subjected to the exposure of a second segment of ileum after which no additional paracenteses were needed The remaining 7 animals at no time required paracentesis One died 3 weeks after eversion of the ileal segment of intestinal obstruction attributable to ingestion of sawdust Autopsy revealed complete absence of fluid within the peritoneal cavity Six animals have survived for periods of 3 months 4 months 4 months 6 months 6 months and 6 months Palpation of their abdomens has revealed the presence of small quantities of fluid estimated not to exceed 500 cc

Observation at laparotomy of the status of the peritoneal cavity after the continued exposure of ileal mucosa for 2 months revealed that both

THE ABSORPTION OF ASCITIC FLUID FOLLOWING THE EVERSION OF A SEGMENT OF INTESTINAL MUCOSA WITHIN THE PERITONEAL CAVITY*

CHARLES G. NEUMANN, NINA S. BRAUNWALD AND
J. WILLIAM HINTON

Although patients with ascites accumulate fluid within the peritoneal cavity, they retain the ability to absorb fluid from the gastrointestinal tract. This common observation led to the development of the concept that it might be possible to employ the fluid absorbing function of a section of intestine for the purpose of aiding in the absorption of fluid from the peritoneal cavity. For the intestine to function in this fashion its mucosa would have to be exposed within the peritoneal cavity and for all but relatively short term studies the peritoneum would be required to tolerate the continued exposure to the intestinal mucosa.

PRELIMINARY OBSERVATIONS

Several preliminary studies were necessary before undertaking the more definitive aspects of the project. The first of these was directed towards the selection of an appropriate segment of the gastrointestinal tract for eversion within the peritoneal cavity. In 3 dogs 7 inch segments of the colon were removed from the course of the bowel, the continuity of the bowel was reestablished by end to end anastomosis and the segments of colon everted. Although the animals all survived the procedure the massive accumulation of mucus within the peritoneal cavity argued in favor of abandoning the large bowel as useful. When segments of the small intestine were substituted in another group of 3 dogs no such accumulation of mucus was observed. It was therefore considered feasible to employ the small intestine if it could be demonstrated that the mucosa could absorb ascitic fluid. Accordingly ascitic fluid collected from a human subject was introduced into a mechanically isolated segment of ileum or jejunum. On each of 4 trials in dogs upon the introduction of sufficient fluid to fill but not distend the isolated segment of small intestine, it was observed that within 2 hours 50 to 60 per cent of the fluid had been absorbed. Comparable absorption occurred when cerebrospinal fluid or normal saline was substituted for the ascitic fluid, whereas when hypertonic saline was instilled the volume of fluid increased markedly.

METHOD

A total of 27 dogs were employed. Of these 5 were sacrificed in the course of learning a useful method for the exposure of intestinal mucosa within the peritoneal cavity. It was observed in these 5 animals that it was not practical to leave the segment of intestine bearing the exposed mucosa unattached to the parietes, for without such attachment the segment became a contracted mass of tissue subject to spontaneous secondary inversion and removal of the mucosa from exposure to free fluid within the peritoneal cavity. It was also observed that unless the omentum was

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THE COMPARATIVE EFFECTS OF DEXTRAN AND BLOOD IN LETHAL STERILE HEMORRHAGIC PANCREATITIS*

MASAUKI HARA, BERNARD W. THOMPSON AND LOYDE H. HUDSON

The existence of severe hypovolemia accompanying the shock state in fatal experimental acute hemorrhagic pancreatitis instigated a study on the comparative effects of the plasma expander, dextran (Plavolex) and blood on the survival rate. Although various therapeutic agents with differing modes of action have been evaluated in the past in experimental hemorrhagic pancreatitis in dogs, rarely has an objective assessment been made as to the presence of infection or its significance in such studies. The basic importance of this consideration is evident in the light of the observation[1] confirmed by several workers that death in dogs from experimental hemorrhagic pancreatitis induced by the injection of bile into the accessory pancreatic duct can be completely or largely prevented by the adequate administration of certain effective antibiotics, such as aureomycin and terramycin. Moreover, Lane and his co-workers have been able to improve the recovery rate in standard shock producing experiments by bleeding from 11 per cent to 88 per cent by the administration of aureomycin, an outcome seemingly implicating a bacterial factor in the irreversibility of shock in the dog. Thus a prime pre-requisite in the execution of certain types of canine experiments is the recognition and prevention of the dog's peculiar susceptibility under adverse conditions to fulminating infections originating from bacteria which had previously lain dormant.

METHOD

A previously described method[3] of producing a sterile yet highly lethal form of acute hemorrhagic pancreatitis was employed in the investigation. Trypsin (180,000 to 250,000 u. Tryptar) was injected under pressure into the accessory pancreatic duct of healthy mongrels averaging 11 to 17 kg, which were prepared by the oral administration of 1.5 to 3 gm. of terramycin and neomycin† for 3 to 4 days preoperatively. Stool and operative pancreatic specimens were obtained for bacteriological evaluation.

The experiments were divided into two categories:

Group I. Dextran (Plavolex) 35 to 60 cc./kg., 6 per cent in normal saline.
Group II. Blood 35 to 45 cc./kg. and saline 30 cc./kg.

The agent was administered following induction of the pancreatitis over a period of 6 to 10 hours unless death interceded. The animals succumbing were autopsied as promptly as possible using sterile technique and cultures of peritoneal fluid, pancreas, bile and portal blood obtained. The volume of peritoneal fluid was accurately measured. The bacteriological analysis was conducted in an identical manner as in the previous study.

Aside from routine serial amylase and antithrombin determinations, other laboratory examinations were carried out during various phases of

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*From the Department of Surgery, University of Arkansas School of Medicine, Little Rock, Ark. This investigation was supported by U.S.P.H. Grant No. A 438 (C).
The technical assistance of Miss Lavada Phillips, Dr. H. Hardin and Dr. T. H. Oddie is gratefully acknowledged.
†The terramycin was generously supplied by Dr. H. W. Rudel of Pfizer Laboratories and the neomycin by Dr. Harold R. Reames of Upjohn Co.

on gross and miscroscopic examination the mucosa retained its specific characteristics without evidence of retrogressive changes Scattered widely over the peritoneum of all parts of the abdominal cavity were gray pin head sized areas which under the microscope were composed of leucocytes and monocytes In none of the animals examined was there any localized collections of pus but on occasion a few strands of mucinous material were encountered

DISCUSSION

Although it is suspected that the marked decrease in the collection of ascitic fluid in animals subjected to constriction of the inferior vena cava and then to exposure of ileal mucosa within the peritoneal cavity is attributable to continuous absorption of fluid by the mucosa we do not have conclusive evidence that some other process may not be accounting for or contributing to the observed effect Further studies are in progress to elucidate the physiological basis for the control of the experimentally produced ascites to learn whether segments of small intestinal mucosa other than of ileum may be more effective and to ascertain what may be the most appropriate length of intestinal mucosa needed to produce the desired effect What seems apparent to date is that dogs are able to tolerate the continued exposure of their peritoneal cavities to intestinal mucosa for periods of time up to 6 months

SUMMARY

Of 14 dogs made ascitic by constriction of the inferior vena cava 6 were set aside as controls and in these massive ascites persisted for 6 months Eight were subjected to the exposure within the peritoneal cavity of the mucosa of a segment of ileum In these the ascites either failed to recur or recurred in markedly reduced quantities

REFERENCES

1 McKee F W Schilling J A Tishkoff G H and Hyatt R F Experimental ascites Effects of sodium chloride and protein intake on protein metabolism of dogs with constricted inferior vena cava Surg Gyn Obst *89* 529 1949

2 Schilling J A McCoord A B Clausen S W Troup S B and McKee F W Experimental ascites Studies of electrolyte balance in dogs with partial and complete occlusion of the portal vein and of the inferior vena cava above and below the liver J Clin Invest *31* 702 1952

have exerted a deleterious effect by overloading the circulation

In 5 additional experiments 35 cc dextran/kg was administered in a 4 to 5 hours period following production of the pancreatitis During the infusion period the hematocrit fell or remained stationary but had risen 11 per cent within 4 hours of the post infusion period The peak of the serum dextran level of 4 to 5 mg comparable to similar figures for controls was attained shortly after completion of the infusion but decreased approximately 50 per cent at 4 hours at a more accelerated rate than in the controls After 1 hour levels of dextran exceeding 1 mg could be detected in the peritoneal fluid and at the end of 4 hours approximated 50 per cent of the corresponding serum value However the quantitative loss of dextran could not be ascertained in the absence of plasma volume determinations The largest volumes of peritoneal fluid totaling 1200 to 1500 cc in some instances were recorded in this group

Blood Series. Whole blood therapy produced a significant rise in the number of survivors During the initial 6 to 8 hours the animals appeared as critically stricken as those in the preceding categories but many rallied visibly with sustained slowing and strengthening of the pulse Two of the 7 fatalities occurred quickly within a period of 3½ hours of an intractable shock state apparently refractory to the infusion of blood

DISCUSSION

The presence of a severe degree of hypovolemia in experimental pancreatitis due to trypsin appears irrefutable a fact previously established in relation to experimental bile pancreatitis by Elliott[5] and in clinical acute and recurring pancreatitis by Keith and Watman[6]

The inability of the plasma expander dextran to provide greater protection to the animals seems explainable on the basis of the leakage of the colloid into the peritoneal cavity due to increased capillary permeability resulting in the failure to maintain a critical expansion of the plasma volume The observations recorded in the study the disappearance of dextran from the circulation at a more accelerated rate than the controls and its recovery in the peritoneal fluid the delayed rise in the hematocrit indicative of continuing loss of plasma and the rapid transit time of radioactive globulin through the capillary barrier into the peritoneal cavity tend to confirm this impression However before a more affirmative stand can be taken on this view additional data concerning the plasma and blood volumes are necessary

In an admirably conceived and executed study Davis and his co-workers[7] have recently presented convincing evidence that dextran is unable to maintain expansion of the plasma volume in hypovolemia associated with increased capillary permeability Among other experiments using a standard thermal burn in dogs as a prototype of hypovolemia with increased capillary permeability they demonstrated a fall of 59 per cent in the plasma volume and the loss of 70 per cent of the infused dextran from the circulation at the end of 4 hours

Our observations are seemingly at variance with those of Elliott[5] who found that the administration of 20 cc /kg of dextran yielded 4 survivors out of 6 mongrels afflicted with bile induced pancreatitis Concentrated human albumin (5 to 7 cc /kg) proved to be even more efficacious result

the investigation. Plasma and blood volume estimations were performed using dye T-1824 and/or radioactive iodinated globulin in 5 control pancreatitis experiments. The dextran content of blood and peritoneal fluid was determined by the method of Metcalf and Rousselot[4] in 5 of the dextran experiments. Unfortunately the tests most pertinent to the investigation, plasma and blood volume measurements using the R.I.H.S.A. method, could not be carried out as planned.

RESULTS

In order to define the role of infection as a factor influencing the outcome, the experiments were defined as sterile or infected on the basis of the bacteriological survey of the peritoneal fluid and pancreas cultures from necropsy.

The clinical and pathological features of trypsin-induced pancreatitis have been related in a previous communication.[3] In essence the clinical course was that of progressive shock. The pancreas at necropsy had been converted into a swollen, dark red hemorrhagic mass possessing in areas a gelatinous consistency due to extreme necrosis. The peritoneal cavity usually contained 500 to 1000 cc. of grossly bloody fluid with a hematocrit value of 1 to 12 per cent. These findings clearly revealed the loss of substantial quantities of blood and plasma into the peritoneal cavity and into the pancreas and peripancreatic region. The over-all picture contrasted *strikingly with the more benign process observed in pancreatitis incited by bile.*

There was a decrease averaging 35 to 40 per cent in plasma volume and approximately 25 per cent in blood volume at the end of 6 hours, but the variations in the survival time of the 5 experiments selected for the studies did not permit a valid over-all average value. For example, 1 animal expired in 3 hours with a 39 per cent decrease in the plasma volume and a second after 1½ hours with a 28 per cent decrease. In 2 experiments 15 per cent and 22 per cent respectively of the administered radioactive globulin was recovered in the peritoneal fluid aspirated 2 hours following induction of the pancreatitis. At the termination of the experiment over 90 per cent of the globulin loss could be accounted for in the peritoneal exudate. The hematocrit rose 10 to 20 per cent after 6 hours.

Dextran Group. The mortality rate of 71 per cent did not reflect an appreciable improvement over the control figure of 90 per cent. The average survival time was lengthened to 16½ hours. Although increasing amounts of the plasma expander were administered in an effort to boost the survival rate, it appeared that amounts in the range of 60 cc./kg. may

Table 1. Mortality Rate in Pancreatitis Treated with Dextran and Blood

	NO. OF DOGS	MORTALITY	NO. OF STERILE PANCREATITIS	MORTALITY IN STERILE PANCREATITIS
Controls (saline)	23	21 (91%)	16	88%
Dextran	19	11 (71%)	14	71%
Blood (and saline)	21	7 (33%)	6	29%

THE SELECTIVE LOCALIZATION OF ALKALOIDS IN PANCREATIC TISSUE*

George L. Nardi and John H. Smith

Selective tissue localization of chemical compounds has been a long sought though elusive goal in clinical medicine. Since Ehrlich's quest for a magic bullet investigators have attempted to predict and produce compounds that have specific action on specific limited portions of the living organism. Those few successes that have been discovered have been useful as powerful tools in almost all phases of investigation concerning the systems to which they apply. Perhaps the most outstanding example of such specificity and its usefulness is the familiar relation of iodine to the thyroid gland. The value of such an agent with specificity for the pancreas needs no discussion. Our experiments indicate that certain plant alkaloids extractable from the herb colombo root will localize in the pancreas of rats and mice. If a similar behavior can be demonstrated in man these compounds should be of value in elucidating the oft bizarre manifestations of pancreatic disease.

Colombo root is an herb indigenous to Africa and most easily and inexpensively obtained from the herb stores to be found in the Oriental sections of our larger cities. By a simple process of alcoholic extraction, precipitation and crystallization numerous alkaloids may be obtained. The formulae of some of these are illustrated in Fig. 1. These compounds are highly colored and give striking fluorescence under ultraviolet light. They are frequently isolated as the tetrahydro derivatives but it is doubtful that these exist naturally in the plant.[1]

Previous work with some of these alkaloids demonstrated that parenteral and intravenous injection resulted in marked fluorescence of the pancreas of mice and rats when the viscera of these animals were examined by ultraviolet light at autopsy. Since fluorescence is a physical phenomenon it is not proof of quantitative local concentration.

METHOD AND RESULTS

To determine accurately the extent of selective pancreatic localization of one of these alkaloids the picrolonic acid precipitation method of Richter[3] was utilized to analyze quantitatively the pancreatic alkaloidal content of rats injected with a uniform dose of berberine. This method yielded better than 95 per cent recovery of known amounts of the alkaloid in our hands. Berberine was chosen as the alkaloid to be studied not only because it had shown a brilliant pancreatic fluorescence after injection but because it was both easily available commercially and because it had yielded highly accurate results with the analytical method to be utilized.

Twenty two rats were injected subcutaneously with a uniform dose of 75 mg./kg. of berberine hydrochloride. They were sacrificed after 4 to 6 hours and the viscera inspected under a mercury vapor lamp. The brilliant yellow fluorescence characteristic of berberine was seen in the pancreatic

*From the Department of Surgery, Harvard Medical School and the Surgical Services of the Massachusetts General Hospital, Boston, Mass. This work was supported by a grant from the American Cancer Society.

ing in the recovery of all the animals Previous reference has been made to the fact that certain antibiotics can similarly circumvent death indicating that the dog can compensate for the degree of hypovolemia incurred in bile incited pancreatitis provided an overwhelming infection is not superimposed Evidently the primary consideration in the recovery of the animal from this form of pancreatitis is the prevention of a fulminating infection most frequently clostridial by averting shock, or adequate protection with antibiotics The pancreatitis produced by trypsin is a more devastating one attended with a greater loss of blood than that due to bile, a factor which in all probability accounts for the discrepancy concerning the efficacy of dextran

The superiority of blood over dextran suggests that there frequently exists a critical decrement in the blood volume in trypsin pancreatitis although the quantity of blood administered would appear to be inadequate replacement for severe deficits Likewise the failure of blood transfusions to salvage an appreciable number afflicted with trypsin pancreatitis would implicate other factors, besides hypovolemic shock and infection in their demise Additional studies are needed to elucidate these and other aspects of the pancreatitis problem Our results in 21 experiments on the use of an antitryptic substance with and without the adjunct of blood therapy suggests that a combination of the two may be more effective than blood alone

SUMMARY

1 Whole blood therapy appears to be more efficacious than dextran in increasing the survival rate of lethal trypsin induced pancreatitis

2 The ineffectiveness of dextran seems to result from a loss of colloid into the peritoneal cavity in the presence of increased capillary permeability

3 The mortality of 30 per cent with the use of blood suggests that hypovolemic shock may not be the sole mechanism concerned in the lethal outcome of experimental pancreatitis

REFERENCES

1 Persky L Schweinburg F Jacob S and Fine J Aureomycin in experimental acute pancreatitis of dogs Surgery *30* 652 56 1951

2 Fine J Frank H Schweinburg F Jacob S and Gordon T The bacterial factor in traumatic shock Ann N York Acad Sc *55* 429-45 1952

3 Hara M Baber J C Millar P H and Hardin H Experimental hemorrhagic pancreatitis I A method of production of non infected lethal hemorrhagic pancreatitis in Surgical Forum 1954 Philadelphia W B Saunders Co 1955 p 395

4 Metcalf W and Rousselot L M A simple accurate and rapid method for the quantitative determination of dextran in blood and urine J Laborat Clin M *40* 901 06 1952

5 Elliott Dan W The mechanism of benefit derived from concentrated human serum albumin in experimental acute pancreatitis in Surgical Forum 1954 Philadelphia W B Saunders Co 1955 p 381

6 Keith L M and Watman R N Blood volume deficits in pancreatitis in Surgical Forum 1954 Philadelphia W B Saunders Co 1955 p 380

7 Davis J H Benson J W Wolfe M Nelson B and Abbott W E The effect of capillary permeability on the maintenance of plasma volume following the administration of dextran and albumin in Surgical Forum 1954 Philadelphia W B Saunders Co 1955 p 455

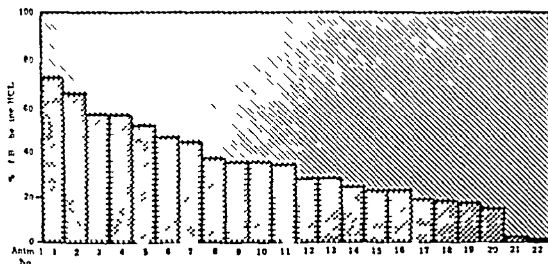


Fig —

in the left vertical column all have a similar nuclear structure and differ from each other only in their side chains The compounds in the right vertical column all have an identical nuclear configuration which differs from the first column only in the saturation of the second phenolic ring i e they are tetrahydro derivatives

It will be noted that palmatine and berberine differ only in the side chain where in berberine formaldehyde acetal (methylenedioxy group) occurs rather than the methyl ethers found in palmatine Physically these compounds are quite similar bright yellow needles with a brilliant yellow fluorescence under ultraviolet light Chemically their reactions and solubilities are quite similar [1]

Reference to the formulae for tetrahydropalmatine and tetrahydroberberine indicates that these compounds differ from their parent compounds in the saturation of one ring of the nucleus but are quite similar to each other in structure These compounds are likewise quite similar occurring as pale greenish yellow almost colorless platelets with a brilliant bluish white fluorescence Their chemical reactions and solubilities are quite similar [1]

Thus berberine and its reduced form and palmatine and its reduced form differ greatly physically and chemically

Palmatine was synthesized from berberine by the method of Spath and Quitensky[4] and Spath and Messetig[5] and was independently synthesized from tetrahydropalmatine by the method of Haworth Kopfli and Perkin [6] The two products were identical

Tetrahydroberberine was prepared from berberine by reduction with zinc metal in glacial acetic acid These compounds were then injected intravenously into rats using a dose of 20 mg /kg Results are summarized in Fig 3 wherein the outline of a pancreas is affixed next to the formula of the alkaloid injected The blackened pancreas indicates pancreatic localization A clear outline indicates failure of localization

As can be seen berberine palmatine and colombamine all compounds with an unsaturated nuclear structure but with differing side chains all

ALKALOIDS OF COLOMBO ROOT

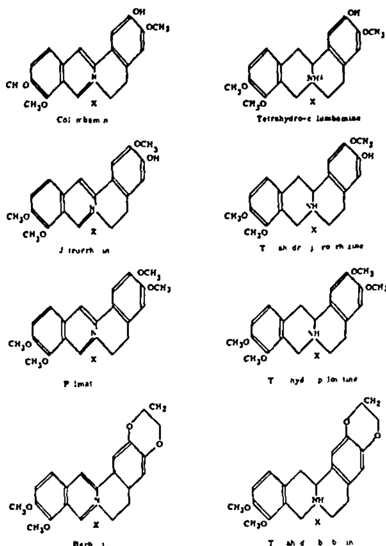


Fig 1

tissue and at the site of injection No other fluorescence was noted in any other tissue Control animals not injected with alkaloid showed no similar fluorescence

The pancreatic tissue of these animals was then excised and after histological confirmation of its nature was analyzed quantitatively for berberine The tissue of the control rats yielded a negligible amount of precipitate Twenty of the 22 berberine injected animals were found to have a high percentage of the injected dose in the pancreas (Fig 2)

Having established the localization of berberine in the pancreas some of the other related alkaloids were prepared to evaluate their organ selectivity as well as to study the possible relationship between chemical structure and tissue localization

For this phase of the study the qualitative observation of pancreatic fluorescence was used as indication of localization rather than quantitative estimation of the amount extractable from pancreatic tissue

Reference to the formulae in Fig 1 will make it clear that the alkaloids

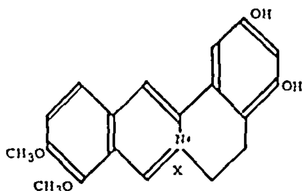


Fig 4

REFERENCES

1 Henry T A The Plant Alkaloids Philadelphia Blakiston Co 1949 4th Ed
2 Taylor I Personal communication
3 Richter P Berberine and its estimation Arch Pharm Berl 252 192 205 1914
4 Späth E and Quietensky H Synthesen des oxy berberins des palmatins und des tetra hydrojatrorrhizine Ber Deut chem Ges 58 2267 2272 1925
5 Späth E and Mosettig E Ueber alkaloide von corydalis cava Synthese des d tetra hydropalmatins Ber Deut chem Ges 59 1496 1500 1926
6 Haworth R D Koepfli J B and Perkin W H Jr A new synthesis of oxoberberine and a synthesis of palmatine J Am Chem Soc 1 518 544 1927

FAT AND NITROGEN METABOLISM IN PATIENTS WITH MASSIVE SMALL BOWEL RESECTIONS*

Morton K Schwartz Albert Medwid Kathleen E Roberts
Marvin Sleisenger and Henry T Randall

Survival after resection of large segments of human small intestine has been reported by many investigators[1] Although there are a large number of case reports of small bowel resections metabolic studies are few and usually represent several metabolic periods in a single patient[2,3] From the few metabolic studies in the literature it has been concluded that a fat and nitrogen absorption defect exists after massive intestinal resection The purpose of the present study was to define more exactly the fat and nitrogen absorption defects in a series of patients with small bowel resections In addition the effects of various drugs and dietary procedures on fat and nitrogen absorption were evaluated in an effort to help the

*From the Sloan Kettering Institute Section of Surgical Metabolism Division of Experimental Surgery and Sloan Kettering Division Cornell University Medical College the Departments of Surgery and Medicine Memorial Center New York and the Department of Medicine New York Hospital This investigation was supported by a research grant (C 1443) from the National Cancer Institute of the National Institutes of Health Public Health Service

The authors wish to express their appreciation to Richard Brassfield M D who referred patient L S to us and to Theodore Miller M D and William Amoroso M D for referring patient J A

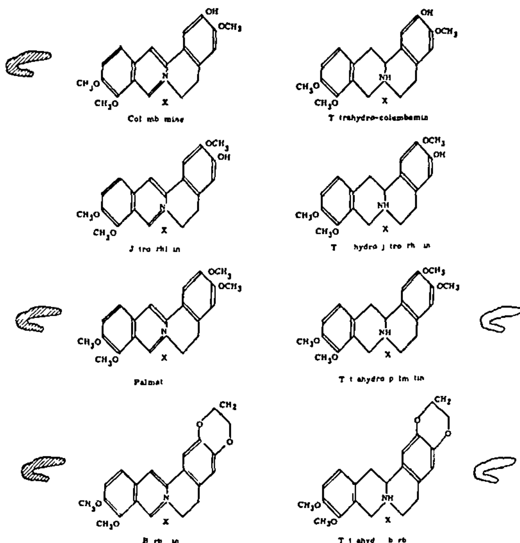


Fig 3

yielded brilliant *in vivo* pancreatic fluorescence after parenteral injection Tetrahydroberberine and tetrahydropalmatine with an unsaturated nuclear structure failed to reveal any pancreatic fluorescence

DISCUSSION

These studies although not complete suggest that the nuclear structure of these compounds rather than the side chains are the important factor *in pancreatic localization It is suggested that this localization is* dependent on the presence of the highly aromatic nuclear structure of Fig 4 We are currently attempting to iodinate this nuclear structure in the hope of achieving *in vivo* opacification of the pancreas

SUMMARY

Certain plant alkaloids demonstrate selective pancreatic fluorescence when injected into rats and mice Pancreatic localization of one of these alkaloids, berberine has been demonstrated by quantitative chemical analysis Of these related alkaloids only those having an unsaturated nucleus showed such specific localization irrespective of side chains

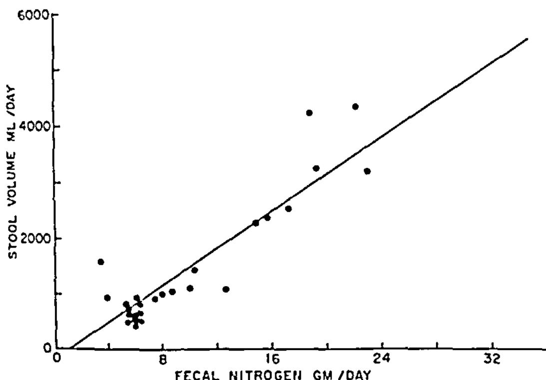


Fig 2 Relationship between fecal nitrogen and stool volume in patient M R

used Carmine markers given at the beginning and end of the metabolic periods divided the stools Urine was collected under toluene and pooled in 3 day collections Fat was determined according to the method of Saxon[5] on aliquots of homogenized stool and diet Stool urine and diet were analyzed for nitrogen by micro Kjeldahl distillation after selenium sulfuric acid hydrogen peroxide digestion

RESULTS AND DISCUSSION

Patient J A who had only a small segment of duodenum remaining after operation was kept alive by both oral and parenteral alimentation The fecal nitrogen of this patient was always as great or greater than the oral nitrogen intake A positive nitrogen balance was obtained on occasion when 2 liters of 5 per cent glucose with 5 per cent protein hydrolysate (amigen) were administered parenterally Despite the fact that this patient did not seem to absorb oral nitrogen there appeared to be about a 50 per cent absorption of fat when the patient received oral feedings During the study the patient was in a cumulative negative nitrogen balance and continued to lose weight In Figure 1 are plotted the cumulative weight losses and the cumulative protoplasm or tissue loss which is calculated from the nitrogen balance by multiplying the nitrogen balance by 32[6] The protoplasm loss and the weight loss are very similar In the early part of the study the protoplasm loss is somewhat greater than the weight loss and this may represent overhydration during the early part of the study when this patient was receiving large quantities of parenteral fluids In the middle part of the study the weight loss is greater than the protoplasm loss and this can be explained by dehydration that occurred during an intestinal yeast infection At this time the stool volume was as great

small bowel resection patient overcome the state of gross undernutrition which usually exists after massive intestinal surgery

METHOD

Of the 5 patients included in this study 2 M R and I S received massive small intestinal resections for superior mesenteric thrombosis M R. was studied 3 years after resection of all but 15 cm of jejunum with a jejuno mid transverse colon anastomosis I S had metabolic balance studies 1, 2 4 and 6 months after resection of the entire ileum the ascending colon and all but 90 cm of jejunum In J A, a retroperitoneal fibroma necessitated resection of the entire ileum jejunum and third part of the duodenum with a duodeno-mid transverse colon anastomosis This patient was studied metabolically from operation until death 11 weeks later from viral hepatitis and bronchopneumonia J S was studied 1 year after gangrene and intestinal ulceration probably secondary to x ray treatment of Hodgkins disease necessitated resection of all but 120 cm of intestine The remaining jejunum was anastomosed to the ileum 30 cm from the ileocecal valve This patient died of perforation of the bowel and a retroperitoneal abscess 3 weeks after the completion of a 5 week metabolic study The fifth patient O A was studied 4 months after 100 cm of ileum the cecum and the ascending colon were resected for chronic ulcers associated with aberrant glandular rests Although this small ileal resection cannot be called a massive intestinal resection patient O A is included since it has been reported that the ileocecal area is of prime importance in the absorption of fat[4]

The patients of the present study were confined to a metabolic ward and were fed by a special research diet kitchen All food items were carefully weighed and aliquots of identical diets analyzed in the laboratory Three day metabolic periods preceded by 3 day adjustment periods were

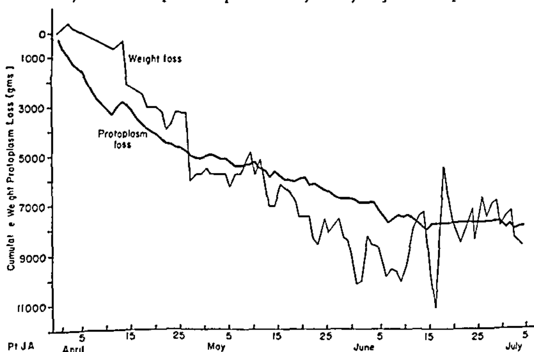


Fig 1 Cumulative weight and cumulative protoplasm losses in patient J A

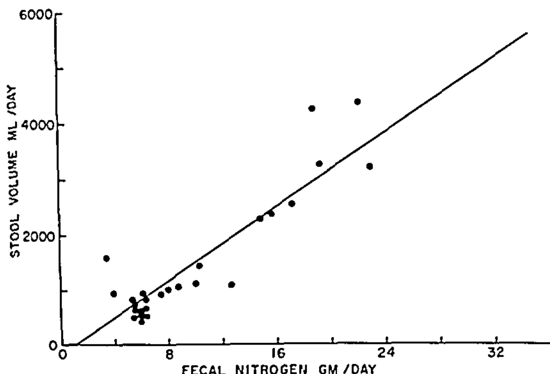


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Patient J A who had only a small segment of duodenum remaining after operation was kept alive by both oral and parenteral alimentation The fecal nitrogen of this patient was always as great or greater than the oral nitrogen intake A positive nitrogen balance was obtained on occasion when 2 liters of 5 per cent glucose with 5 per cent protein hydrolysate (amigen) were administered parenterally Despite the fact that this patient did not seem to absorb oral nitrogen there appeared to be about a 50 per cent absorption of fat when the patient received oral feedings During the study the patient was in a cumulative negative nitrogen balance and continued to lose weight In Figure 1 are plotted the cumulative weight losses and the cumulative protoplasm or tissue loss which is calculated from the nitrogen balance by multiplying the nitrogen balance by 32[6] The protoplasm loss and the weight loss are very similar In the early part of the study the protoplasm loss is somewhat greater than the weight loss and this may represent overhydration during the early part of the study when this patient was receiving large quantities of parenteral fluids In the middle part of the study the weight loss is greater than the protoplasm loss and this can be explained by dehydration that occurred during an intestinal yeast infection At this time the stool volume was as great

Table 1 Effects of Therapeutic Adjuvants on Fat and Nitrogen Absorption

PT AGE SEX	THERAPEUTIC ADJUVANT	FAT gm INTAKE	FAT gm STOOL	PER CENT ABSORBED	NITROGEN gm INTAKE	NITROGEN gm URINE	NITROGEN gm STOOL	NITROGEN gm BALANCE
M R	—	128.3	19.0	62	42.2	9.4	19.3	+13.5
42	Tween 80	128.3	51.7	58	42.2	13.7	17.2	+11.3
M	—	154.2	59.2	62	31.9	21.1	3.5	+ 7.3
	Pancreatin	154.2	70.0	55	36.0	26.3	7.6	+ 2.1
	Banthine	151.2	62.8	59	31.9	25.3	6.1	+ 3.5
	Folic Acid	154.2	63.7	59	31.9	21.3	6.1	+ 4.5
	Probanthine	116.3	49.0	58	38.8	21.0	6.3	+11.5
	—	78.7	39.7	50	35.7	21.6	5.6	+ 8.5
	Cortisone	78.7	35.1	55	35.7	18.1	6.1	+11.5
J S	—	98.4	42.4	57	16.6	7.0	4.9	+ 4.7
44	Pancreatin	98.4	38.6	60	17.2	6.9	5.0	+ 5.3
M	Trypsin	98.4	45.3	53	16.6	3.3	7.3	+ 6.0
	—	101.4	18.0	53	16.2	6.0	7.0	+ 3.2
	Bentyl	101.4	65.6	36	16.2	7.0	5.0	+ 4.2
	Cortisone	100.9	65.6	35	16.0	5.2	7.3	+ 3.5

as 8 liters per day It appears that in general the weight losses in this patient represented protoplasm loss

Patient M R who had all but 45 cm of jejunum removed because of mesenteric thrombosis secondary to Buerger's disease absorbed only 44 to 60 per cent of his dietary fat intake and lost large amounts of nitrogen in his stool The fecal nitrogen was as great as 23.1 gm per day on a nitrogen intake of 12 gm On this nitrogen intake a normal individual would excrete about 3.0 gm of stool nitrogen The fecal nitrogen in this patient was directly related to the stool volume This relationship is shown in Figure 2 and represents 26 three day metabolic periods The nitrogen rose and fell paralleling the changes in stool volume There appeared to be little relationship between nitrogen intake and fecal nitrogen but rather a relationship between stool volume and stool nitrogen

Many therapeutic agents were evaluated in this patient in an attempt to increase absorption Tween 80 (an emulsifying agent) pancreatin banthine probanthine folic acid and cortisone were used None of these had any appreciable effect on fat or nitrogen absorption (Table 1) The only regimen that appeared to help this patient gain weight was a high protein high caloric frequent feeding diet Then if the fecal losses were half of the intake there would still be sufficient absorption to maintain body metabolism Patient M R who was in a poor nutritional state when he started the study was placed on a diet containing over 5000 calories and 260 gm of protein On this diet he gained 13 kg in 6 weeks

Patient J S who had 120 cm of intestine remaining after surgery absorbed from 35 to 66 per cent of the dietary fat and lost large quantities of nitrogen in the stool Pancreatin trypsin bentyl and cortisone were without effect on nitrogen and fat absorption (Table 1)

Fecal fat and nitrogen losses were also excessive in patient L S who had only 90 cm of jejunum remaining after surgery This patient absorbed from 40 to 65 per cent of the fecal fat and the stool nitrogen varied from

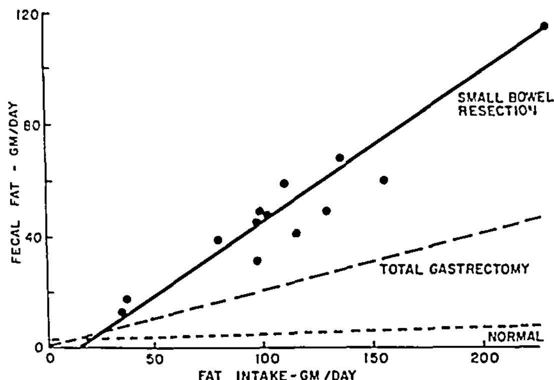


Fig 3 Relationship between fecal fat and dietary fat intake in patients with massive small bowel resections total gastrectomies and in normal persons

1 9 gm on an intake of 2 2 gm to 7 7 gm on an intake of 12 9 gm per day In a study 1 month after surgery only 10 per cent of the fat intake was absorbed A study 2 months after surgery indicated an absorption of 62 per cent of the fat intake and 65 per cent of the fat intake was absorbed 6 months after operation This patient gained 6 kg in the 6 month period after operation This weight gain was accomplished by the use of a frequent feeding high protein moderate fat diet

Patient O A had only a small amount of ileum removed at surgery This patient demonstrated normal fat and nitrogen absorption On a nitrogen intake of 11 8 gm the fecal nitrogen was 2 8 gm per day The fecal fat was only 6 3 gm on an intake of 69 1 gm per day an absorption of 91 per cent

Fecal fat was not related to stool volume but had a definite relationship to fat intake In Figure 3 is shown the relationship between fat intake and fecal fat in normal persons totally gastrectomized individuals and persons with massive small bowel resections This relationship in the present study is based on 14 three day metabolic periods in 3 patients (M R J S and L S) The dietary fat intakes varied from 30 to 227 gm per day The significance of this relationship can be expressed in the following manner On an intake of 100 gm of fat per day a normal person will absorb about 95 gm of fat a totally gastrectomized individual an average of 78 gm of fat and the person with a small bowel resection about 55 gm of fat Since the per cent absorption remains the same if the intake is increased to 200 gm of fat the normal individual will absorb about 190 gm the

SUMMARY AND CONCLUSIONS

totally gastrectomized person about 156 gm, and the person with a small bowel resection an average of 110 gm of fat

The defects in fat and nitrogen absorption after massive small bowel resection are severe The patients in the present study who had massive intestinal resections absorbed only about half of the dietary fat intake and lost large amounts of nitrogen in the stool Therapeutic adjuvants including tween 80 probanthine banthine pancreatin folic acid trypsin bentyl and cortisone did not appear to have any effect in lessening the large fecal losses However a high protein high caloric frequent feeding diet enabled the patients to whom it was given to gain weight and to maintain a good state of nutrition despite large losses

REFERENCES

1 Haymond H E Massive resection of the small intestine an analysis of 257 collected cases Surg Gyn Obst *61* 693 705 1935
2 Althausen T L Uyeyama K and Simpson R G Digestion and absorption after massive resection of the small intestine utilization of food from a natural versus a synthetic diet and a comparison of intestinal absorption tests with nutritional balance studies in a patient with only 45 cm of small intestine Gastroenterology *12* 795 807 1949
3 Jackson W P U and Linder G C Small gut insufficiency following intestinal surgery a clinical and metabolic study of a man surviving with 7 inches of small intestine S Afr J Clin Sci *2* 70 112 1951
4 Kremen A J Linner J H and Nelson C H An experimental evaluation of the nutritional importance of proximal and distal small intestine Ann Surg *140* 439 447 1954
5 Saxon G J A method for the determination of the total fats of undried feces and other moist masses J Biol Chem *17* 99 102 1914
6 Reifenstein E C Jr Albright F and Wells S L The accumulation interpretation and presentation of data pertaining to metabolic balances notably those of calcium phosphorus and nitrogen J Clin Endocr Metab *5* 367 395 1945

Endocrine Aspects of Cancer Dependence Experimental and Clinical Studies

STRESS STUDIES IN ADRENALECTOMIZED PATIENTS*

HARVEY KRUEGER JERREL W BENSON C JACKSON RAYBURN
WILLIAM E ABBOTT STANLEY LEVEY AND WILLIAM D HOLDEN

Ingle and associates[1,2] demonstrated that adrenalectomized force fed rats given a constant dose of adrenal cortical extract responded in the same manner to surgical stress (fractures of the hind limbs) as rats with intact adrenal glands The magnitude of the responses were comparable for the 2 groups of rats and consisted of retention of sodium and chloride and increase in urinary potassium and non protein nitrogen When adrenalectomized rats maintained with saline instead of cortical extract were stressed in the same manner the metabolic responses did not occur These experiments have been cited in subsequent publications to illustrate the hypothesis that the cortical hormones have a permissive activity in their relationship to the metabolic responses which follow stress The hypothesis of permissive action postulates that the cortical hormones are necessary for the metabolic responses to occur but that these responses are not the direct result of the increase in adrenal gland secretion following stress Since methods were not available at that time Ingle s experiments did not include measurements of blood corticoid concentrations Recently Steenburg and his associates[3] have demonstrated an increase in the concentration of blood 17 hydroxycorticoids in surgically stressed adrenalectomized dogs given hydrocortisone by infusion over a 1 hour period

The use of bilateral adrenalectomy in the palliative treatment of metastatic carcinoma of the breast affords an opportunity to study the role of the adrenal cortex in the physiology of the human being The purpose of this investigation was to study the metabolic alterations and blood corticoid concentrations of an adrenalectomized patient maintained on a constant dose of adrenal cortical hormone and subjected to a surgical stress

METHOD

Three patients with metastatic carcinoma of the breast (L L K A and M M) were admitted to the Surgical Metabolic Division of the University

From the Department of Surgery Western Reserve University School of Medicine and the University Hospitals of Cleveland Cleveland Ohio This work was supported in part by a grant from the National Institutes of Health U S Public Health Service (A 760(C3) and the John A Hartford Foundation

The authors wish to express their appreciation to Dr George Sayers and Dr Max L Sweat of the Department of Physiology for the blood steroid analyses

Hospitals of Cleveland for study The surgical stresses were comparable in all 3 cases and consisted of excision of a 6 to 10 cm segment of rib containing metastatic carcinoma These rib segments were obtained to determine the influence of adrenalectomy on the uptake of radioactive phosphorus by osseous metastases In addition to the 3 post adrenalectomy stress studies 1 of the patients (M M) was studied during a rib resection performed prior to adrenalectomy The post adrenalectomy studies were not undertaken until the patients had recovered from the bilateral adrenalectomy and a maintenance dose of replacement therapy had been established The daily maintenance replacement therapy for each patient was 10 mg of hydrocortisone 4 times a day

The intake of water, sodium chloride potassium nitrogen and total calories was kept as constant as possible during the study periods The greatest fluctuations occurred in the water intake A general anesthesia was used for the rib resections in 2 of the patients (L L and K A) In order to keep the intake as constant as possible these patients were given nutrients intravenously of a comparable makeup to their oral diet on the days of operation and 1 to 2 days postoperatively The patients were given the solutions intravenously for a 2 day period prior to operation in order to evaluate the effect of parenteral alimentation Local anesthesia was used in patient M M therefore she was kept on oral feedings during the entire study period In order to eliminate variation in the rate of absorption of hydrocortisone from the gastrointestinal tract on a control day and on the day of operation the maintenance dose was given intravenously over 24 hours by a constant infusion of 1000 ml of 5 per cent dextrose in water containing 10 mg of hydrocortisone Blood corticoid concentrations were determined on each of these days

All nutrients (oral and parenteral) and all excreta (urine feces vomitus)

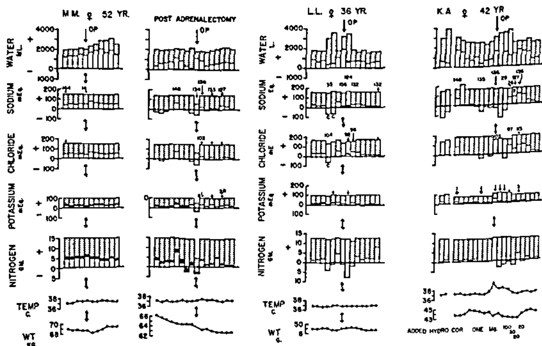


Fig 1

Fig 2

were measured and analyzed for sodium, chloride, potassium and nitrogen according to methods which have been previously described[4] Plasma concentrations of sodium chloride and potassium, and blood concentration of urea nitrogen were determined periodically[4] The peripheral venous blood concentration of hydrocortisone was determined according to the method of Sweat[5]

RESULTS

Figures 1 and 2 show the metabolic balance data obtained from the 3 patients These balance studies are plotted by showing the scale for the intake and the balance per 24 hours on the ordinate and the scale for time on the abscissa The entire intake is plotted from the horizontal zero line upward and the total output plotted downward from the top of the intake column Positive balance is shown as the white area above the zero line and degree of negative balance is represented by the extent of the columns below this line The periods of oral intake are designated by black diagonal lines on white and the periods of total intravenous feeding are indicated by black dots on white Feces are indicated by the solid black areas The heavy arrows indicate the day on which the rib resection was performed No attempt was made to measure insensible fluid losses The numbers above the column are the plasma concentrations of sodium potassium or chloride on the morning of the indicated day The letter C below the columns in Figure 2 indicate the control periods of total intravenous intake At the bottom of each chart are plotted the temperature and daily weight Blood corticosteroid concentrations fluctuated between the lower and upper limits of normal No correlation appears to exist between the fluctuations in blood steroid concentrations and the metabolic alterations which were associated with the stress

DISCUSSION

Patient M M (Figure 1) The balance data obtained during the 2 study periods (rib resection under local anesthesia with intact adrenals and again after adrenalectomy while on a maintenance dose of hydrocortisone), indicate that the stress was not of sufficient magnitude in this patient to invoke any demonstrable metabolic alterations

Patient L L (Figure 2) During the 2 day control period when water and nutrients were given intravenously the daily fluid intake and urine volume were significantly greater than that during the preceding 3 days Although the intakes of sodium chloride and nitrogen were comparable for the 2 day control period and the preceding 3 days there was a significant increase of sodium chloride and nitrogen in the urine when the patient received these substances by intravenous infusion There was no significant difference in potassium excretion during the 2 periods If the balance data for the day of operation and first postoperative day are compared with the data for the 2 control days when the intake was given intravenously the following may be noted (1) The urine volume was significantly decreased on the day of operation (2) The sodium and chloride excretion in the urine was less during the 2 day period of stress than during the control period (3) The amount of nitrogen in the urine was greater during the 2 day period of stress than during the control period (4) There was no significant difference in potassium excretion during the control and stress periods

Patient K A (Figure 2) Because of recurrent episodes of lower urinary

tract infection it was necessary to postpone the rib resection for about 2 weeks after the control intravenous infusion study The patient was given a constant diet before and after the control study Comparison of balance data obtained during these periods which are not shown in the chart with the data for the 2 day control intravenous alimentation period (Fig 2) was essentially the same as previously described for L L Increase in urine volume and urinary losses of sodium chloride and nitrogen with no significant change in potassium was noted The remainder of the chart for K A shows the balance data obtained during an 18 day study period including the day the rib resection was performed During the 4 days prior to operation there were cumulative sodium and chloride deficits of 117 and 70 mEq respectively Potassium and nitrogen balances were in equilibrium during this period The fever which occurred during the stress period was ascribed to a recurrence of the urinary tract infection During the day of operation and the first 2 postoperative days there was a progressive development of the clinical signs and symptoms of adrenal insufficiency (malaise anorexia nausea, vomiting and fall in blood pressure) The patient s plasma volume decreased from 2106 ml prior to operation to 1720 ml on the second postoperative day The electrolyte balance data for the first 2 postoperative days are comparable to those of the control period of intravenous alimentation However there was a significant increase of nitrogen in the urine during the first 2 postoperative days The patient was given additional hydrocortisone from the third through the sixth postoperative days (Fig 2) because of adrenal insufficiency

Our studies and those of others[6, 7] demonstrate that complete withdrawal of adrenal cortical hormones from an adrenalectomized animal or human being is usually followed by a decrease in serum sodium concentration and an increase in serum potassium concentration These changes cannot be explained solely by the external losses of these substances The internal shifts of water sodium and potassium are probably of greater significance in the development of these alterations of serum electrolyte concentrations than increased renal excretion of sodium It has been demonstrated in adrenalectomized animals[8] or men that there is no accelerated rate of protein catabolism when adrenal cortical hormones are completely withdrawn

While the electrolyte balance data following stress are different for L L and K A both of these patients had a decrease in their plasma sodium concentrations and an increase in their plasma potassium concentrations (Fig 2) They also had significant increases in the nitrogen content of the urine A reasonable interpretation of the metabolic alterations demonstrated by these 2 patients is that following the stress (1) they were deficient in the adrenal cortical steroid or steroids that primarily effect electrolyte metabolism K A probably having had a greater deficiency than L L since the magnitude of her stress was increased by a postoperative urinary tract infection and (2) they had a sufficient amount of adrenal hormone which primarily influences nitrogen metabolism to permit an increase in the nitrogen content in the urine

Consistent with this interpretation is the fact that they were given only hydrocortisone an adrenal cortical steroid which has slight activity with respect to mineral metabolism but markedly influences nitrogen metabolism It is presumed that prior to the stress the slight activity of hydrocortisone on electrolyte metabolism was sufficient to maintain the patients in an appar

ently normal state Ingle was able to demonstrate permissive action of adrenal steroids on both electrolyte and nitrogen metabolism since his adrenalectomized rats were maintained with adrenal cortical extract which contains significant amounts of salt retaining substances

SUMMARY

1 Stress studies in 3 adrenalectomized patients maintained on a constant dose of adrenal cortical steroid are described

2 The metabolic alterations which occurred in 2 of the patients are compatible with a deficiency of adrenal hormone that primarily influences electrolyte metabolism These metabolic changes at the same time demonstrate the permissive activity of adrenal cortical steroids in their relationship to alterations of nitrogen metabolism following stress This probably occurred because the adrenal cortical steroid (hydrocortisone) which was used for replacement therapy has slight activity with respect to electrolyte metabolism but markedly influences nitrogen metabolism

REFERENCES

1 Ingle D J, Ward F O and Kuizenga M H The relationship of the adrenal glands to changes in urinary non protein nitrogen following multiple fractures in the force fed rat Am J Physiol *149* 510 521 1947

2 Ingle D J, Meeks R C, and Thomas K E The effect of fractures upon urinary electrolytes in non adrenalectomized rats and in adrenalectomized rats treated with adrenal cortex extract Endocrinology *49* 703 708 1951

3 Steenburg R W and Canong W F Observations on the influence of extra adrenal factors on circulating 17 hydroxycorticoids in the surgically stressed adrenalectomized animal Surgery *38* 92 103 1955

4 Abbott W E Krieger H Babb L I Levey S and Holden W D Metabolic alterations in surgical patients I The effect of altering the electrolyte carbohydrate and amino acid intake Ann Surg *138* 434 452 1953

Sweat M L Silica gel microcolumn for chromatographic resolution of cortical steroids. Anal Chem 26 1964 1967 1954

6 Mendelsohn M L and Pearson O H Alterations in water and salt metabolism after bilateral adrenalectomy in man J Clin Endocr Metab *15* 409-423 1955

7 Harrop C A The influence of the adrenal cortex upon the distribution of body water Bull Johns Hopkins Hosp 59 11 24 1936

8 Bondy P K and Engel F L Prolonged survival of adrenalectomized nephrectomized rats on a low potassium diet Proc Soc Exp Biol N Y *66* 104 107 1947

RELATIONSHIP OF EXPERIMENTAL RENAL DAMAGE TO TOLERANCE OF SURGICAL STRESS*

EMILE L. MEINE, JR.

This study has grown out of the clinical impression that low grade renal damage may exist in some patients whose kidney function tests are normal or borderline[1] but in whom the stress of major surgery precipitates renal failure and death. To achieve a comparable situation experimentally, it was elected to inflict acute renal damage on healthy dogs and then submit them to a major surgical procedure while some degree of renal impairment might still exist, though not be fully apparent.

METHOD

Two methods of producing the initial kidney damage were chosen. The first, renal anoxia, was obtained by 1 hours of bilateral renal artery occlusion.[3,4] A second method consisted of injecting sodium morrhuate (0.08 ml per kg of 2.5 per cent solution) directly into each renal artery. Both methods required an initial laparotomy, therefore dogs of a control group had a similar "sham operation" initially. Nine or 10 days later, after allowing time for partial recovery, a second operation designed to inflict a heavy operative load was performed on all surviving dogs. This consisted of cholecystectomy, splenectomy, bleeding of 18 cc. blood per kg. body weight, and unilateral nephrectomy. This is referred to subsequently as the "stress operation." It includes the factors of anesthesia, surgical trauma and severe blood loss, all deemed important in the production of acute postoperative renal failure.[5,6,7]

Healthy mongrel dogs (9.2 to 20.0 kg.) of both sexes were used. Sterile surgical operations were done under anesthesia provided by intraperitoneal morphine (30 to 60 mg.) and sodium pentobarbital (20 mg./kg.). No antibiotics were used. Dogs were fed only the standard animal diet. As a measure of renal function through stages of the experiment, blood urea nitrogen (BUN) and non-protein (NPN) determinations were made. These are relatively insensitive tests for minor degrees of kidney damage but were therefore suitable for the present purpose, especially in view of their wide clinical use.

These nitrogen determinations were made before the first operation to establish a base line and then on various days after each operation. The average preliminary levels were considered normal for each dog and subsequent determinations expressed as per cent of normal. Only the BUN data is used in this report. The experiment was concluded 15 days after the stress operation for all surviving animals. Gross anatomical and histological examinations of the kidneys were made at death or sacrifice of the animals and on specimens obtained at the stress operation, with good correlation between the anatomical findings and BUN determinations.

*From the Department of Surgery, University of Illinois College of Medicine, aided by a grant from the Graduate School, University of Illinois, Chicago, Ill.

Credit is hereby extended Ruth McGrath for valuable technical assistance.

RESULTS

Fifty seven dogs were used divided into 3 groups as shown in Table 1 There were no control group deaths after laparotomy but mortality rates of 27 8 per cent and 40 0 per cent were sustained in the animals having renal artery occlusion and injection of morrhuate into the kidney respectively The 2 methods of causing renal damage were therefore similar in severity with respect to mortality Deaths occurred from 3½ to 6 days after artery occlusion and from 2 to 6 days after morrhuate treatment The degree of azotemia at the time of death ranged from 125 to 1810 per cent of normal BUN All dogs that survived the first procedure were stress operated 9 or 10 days later Under each group of dogs 2 subdivisions will be considered post stress deaths and post stress survivors The median BUN levels for all the animals of each group are shown for mortalities in Figure 1 and for survivors in Figure 2 Only 1 control dog died post stress while the mortality rates for the other two groups were much higher as shown in Table 1 being 38 5 per cent for the occlusion group and 53 3 per cent for morrhuate treated animals

Table 1 Results of the 3 Groups Through Both Operations

GROUP	DOGS OPERATED	DIED	PER CENT MORTALITY	STRESS OPERATED	DIED	PER CENT MORTALITY
Control	14	0		14	1	7 1
Occlusion	18	5	27.8	13	5	38.5
Morrhuate	25	10	40 0	15	8	53.3

Among the post stress survivors similarity between the 2 treated groups is shown in Figure 2 Occlusion and morrhuate dogs had very sharp elevations of BUN levels for a few days after the stress operation with these groups and the control group becoming closer together by the end of the experiment Histologically both renal artery occlusion and sodium morrhuate injection into the renal artery caused damage chiefly to the tubular portions of the nephron Occlusion also caused interstitial fibrosis and calcification of damaged tubules the changes being widespread Morrhuate injection often caused massive infarction and necrosis especially among the early pre stress deaths When the damage was short of necrosis there was usually a marked inflammatory response with much fibroplasia but rarely any calcification

DISCUSSION

The purpose of this experimental study was to determine whether small to moderate degrees of acute renal damage would alter significantly the mortality after a given stress operation To evaluate this the 3 groups of dogs that were stress operated were divided into subgroups of survivors and mortalities post stress In the *controls* the median BUN for survivors was below normal on the fourth and eighth days after sham operation presumably after dietary intake was low for the first 3 or 4 days The operation itself did not evoke a marked catabolic response and negative nitrogen balance is known to exist in this phase Further these animals had normal kidney function as compared with the other groups After stress however

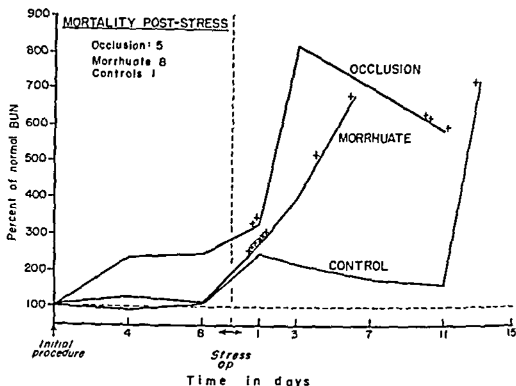


Fig 1 Illustrated above are median BUN levels of the 3 groups of dogs that died after the stress operation Crosses indicate time of deaths Mortality rates following the stress operation were 53.3 per cent for morrhuate 38.5 per cent for occlusion and 7 1 per cent for control groups

Fig 2 Shown above are median BUN levels for the 3 groups of dogs that survived both surgical procedures The pre stress BUN levels were similar for each group of survivors shown here to dogs of the same group that died after the stress operation (Fig 1) Note on both graphs that the morrhuate injected dogs had a relatively normal median BUN level prior to the stress procedure

the values for the 1 dog that died were at or above the upper range of BUN readings for all survivors in this group

In the occlusion group there was the widest range of pre stress BUN values among survivors of any group The medians however were only 166 per cent and 126 per cent of normal BUN on the days (fourth and eighth) that samples were drawn Dogs of this group destined to die post stress showed a more marked pre stress response to occlusion than did the subsequent survivors The medians for the deaths were 231 and 215 per cent so that these dogs seemed to separate themselves in a manner which made their subsequent death more predictable than with similar dogs in either of the other groups The difference between occlusion sub groups was quite marked post stress Median BUN values rose quite sharply on the first and third days post stress for the dogs dying while survivors soon began to improve

The morrhuate dog showed little difference between sub groups in the pre stress period Median BUN levels on the 2 days evaluated pre stress were 109 and 115 per cent for survivors and 129 and 109 per cent for subsequent mortalities Considering these medians it is apparent that BUN determinations immediately before the stress operation were not appreciably abnormal nor was there any difference between the sub groups of subsequent mortalities and survivors This is quite distinct from the occlusion sub groups before the stress operation Thus the morrhuate treated group behaved in a manner which supports the belief that some animals in whom kidney damage had been produced and who are unable to tolerate a heavy surgical stress may yet be relatively asymptomatic prior to stress

There were statistically significant differences between survivors in the treated groups as compared with the control survivors on the first and third post stress days The occlusion animals had median BUN values of 218 per cent and 163 per cent on these days Morrhuate group medians were 205 and 137 per cent while control survivors had only 128 and 90 per cent of normal BUN of the same days While the treated groups differed from the control they did not significantly differ from each other At 15 days post stress the treated dogs still had higher median BUN values although the groups were less clearly separated after the third post stress day The first few days were considered most important in deciding the fate of animals since 40 per cent of the occlusion and 75 per cent of the morrhuate deaths occurred one day post stress

SUMMARY

Two experimental methods of producing kidney damage bilateral renal artery occlusion for 4 hours and the injection of 2.5 per cent sodium morrhuate into the renal circulation were utilized to prepare dogs for a second operation designed to impose a heavy surgical stress The mortality rates in these 2 methods of preparation were comparable Fifty seven animals including a control group were used The survivors of the above procedures and a control group of sham operated dogs were submitted to a stress operation 9 or 10 days later consisting of cholecystectomy splenectomy unilateral nephrectomy and severe bleeding

In the surviving groups subjected to the stress operation there were 11 control dogs 13 dogs which had occlusion of the renal artery and 15 morrhuate treated animals Post stress mortality was 38.5 per cent in the occlu

sion group 53.3 per cent in morrhuate injected dogs and 7.1 per cent for controls. Comparison of the first 2 with the latter group proved the mortality figures to be significant (p = 0.01). The test of renal function selected was the blood urea nitrogen determination (BUN), which has wide clinical application. The occlusion dogs that died after the stress operation generally had higher pre-stress BUN levels than others in that group. Only 20 per cent of the dogs that had previous artery occlusion and subsequently died post-stress had a BUN of less than 112 per cent of normal just before the stress procedure. However, 75 per cent of the morrhuate dogs that died following the stress operation had BUN levels not more than 112 per cent of normal just prior to the stress operation. This finding suggests that renal damage might exist in such a minor degree that it could not be detected clinically but nevertheless would not permit the subject to tolerate a severe surgical stress.

Sodium morrhuate was therefore more useful than renal artery occlusion as an investigative method in this study, and is a suitable agent for producing certain types of renal damage. Support is lent experimentally to the concept that kidney damage not clearly apparent by blood nitrogen studies may exist in otherwise healthy dogs which are unable to tolerate a severe surgical stress.

REFERENCES

1. Newburgh, L. H., and Camara, A. A.: Lack of correlation between symptoms and degree of renal impairment. Ann. Int. M. *35*: 39-44, 1951.
2. Clark, J. K., and Barker, H. S.: The renal problem in surgery. Advances in medicine and surgery from the Graduate School of Medicine of the University of Pennsylvania, 1952, pp. 182-190.
3. Hamilton, P. B., Phillips, R. A., and Hiller, A.: Duration of renal ischemia required to produce uremia. Am. J. Physiol. *152*: 517-522, 1948.
4. Hawthorne, E. W., and Wakerlin, G. E.: Effects of preliminary renal ischemia on experimental renal hypertension. Am. J. Physiol. *177*: 311-317, 1954.
5. Phillips, R. A., Dole, V. P., Hamilton, P. B., Emerson, K., Jr., Archibald, R. M., and Van Slyke, D. D.: Effects of acute hemorrhagic and traumatic shock on renal functions of dogs. Am. J. Physiol. *145*: 314-336, 1946.
6. Moyer, C. A.: Acute temporary changes in renal function associated with major surgical procedures. Surgery *27*: 198-207, 1950.
7. Ariel, I. M., and Miller, F.: The effects of abdominal surgery upon renal clearances. Surgery *28*: 716-728, 1950.

STUDIES ON THE COMPLETENESS OF SURGICAL ADRENALECTOMY*

CHARLES ECKERT, THEODORE L. WEICHSELBAUM AND HARRY MARGRAF

In certain cases of advanced breast cancer the surgical removal of the adrenal glands retards the growth rate of the cancer while in other cases it does not. The reasons for success or failure are unknown. However, the presence of accessory adrenal cortical tissue might explain the lack of effectiveness of this procedure in many cases. Accessory adrenal tissue has been found to be widely dispersed.[1,2] Graham[3] in studying the soft tissues of the preaortic region between the renal and celiac arteries found accessory adrenal tissue in 32 of 100 cases. Despite the frequency of accessory adrenal tissue, proof of the functional capacity of this tissue is lacking.

These studies were undertaken in an attempt to answer the question: Is accessory adrenal tissue functional?

METHOD

Patients with metastatic carcinoma of the breast were observed after bilateral adrenalectomy and oophorectomy. Adrenal function was studied by determining the free and glucuronic acid conjugated 17-hydroxycorticosteroids (17-OH-C) in plasma and urine. The changes in concentrations of the 17-OH-C in plasma and urine associated with ACTH administration with and without glycyrrhizin were determined. The urine and blood samples were collected while the patients were being given cortisone 10 to 50 mg. daily, during a subsequent tolerated period of no cortisone or other substitution therapy (96 hours or less), and in 7 patients while ammoniated glycyrrhizin was being given. The general structure of glycyrrhetinic acid

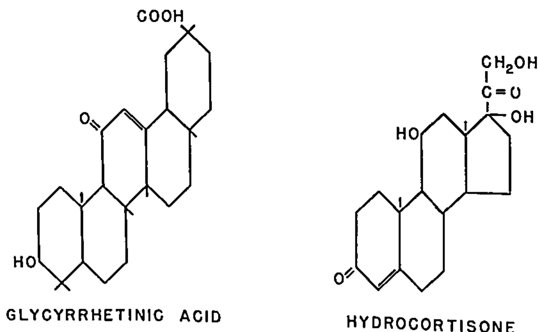


Fig. 1

*From the Department of Surgery, Washington University School of Medicine, St. Louis, Missouri. Aided by a Grant from the American Cancer Society (INSTR 32G).

sion group 53.3 per cent in morrhuate injected dogs and 7.1 per cent for controls. Comparison of the first 2 with the latter group proved the mortality figures to be significant ($p = 0.01$). The test of renal function selected was the blood urea nitrogen determination (BUN) which has wide clinical application. The occlusion dogs that died after the stress operation generally had higher pre stress BUN levels than others in that group. Only 20 per cent of the dogs that had previous artery occlusion and subsequently died post stress had a BUN of less than 112 per cent of normal just before the stress procedure. However, 75 per cent of the morrhuate dogs that died following the stress operation had BUN levels not more than 112 per cent of normal just prior to the stress operation. This finding suggests that renal damage might exist in such a minor degree that it could not be detected clinically but nevertheless would not permit the subject to tolerate a severe surgical stress.

Sodium morrhuate was therefore more useful than renal artery occlusion as an investigative method in this study and is a suitable agent for producing certain types of renal damage. Support is lent experimentally to the concept that kidney damage, not clearly apparent by blood nitrogen studies, may exist in otherwise healthy dogs which are unable to tolerate a severe surgical stress.

REFERENCES

1. Newburgh, L. H., and Camara, A. A.: Lack of correlation between symptoms and degree of renal impairment. Ann. Int. M. *35*: 39-41, 1951.
2. Clark, J. K., and Barker, E. S.: The renal problem in surgery. Advances in medicine and surgery from the Graduate School of Medicine of the University of Pennsylvania, 1952, pp. 182-190.
3. Hamilton, P. B., Phillips, R. A., and Hiller, A.: Duration of renal ischemia required to produce uremia. Am. J. Physiol. *152*: 517-522, 1948.
4. Hawthorne, F. W., and Wakerlin, G. E.: Effects of preliminary renal ischemia on experimental renal hypertension. Am. J. Physiol. *177*: 341-347, 1954.
5. Phillips, R. A., Dole, V. P., Hamilton, P. N., Emerson, K., Jr., Archibald, R. M., and Van Slyke, D. D.: Effects of acute hemorrhagic and traumatic shock on renal functions of dogs. Am. J. Physiol. *145*: 314-336, 1946.
6. Moyer, C. A.: Acute temporary changes in renal function associated with major surgical procedures. Surgery *27*: 198-207, 1950.
7. Arnel, I. M., and Miller, F.: The effects of abdominal surgery upon renal clearances. Surgery *28*: 716-728, 1950.

deviation of 1 5 μg per cent In 17 normal persons the glucuronic acid conjugated 17 OH C averaged 16 8 μg per cent with a standard deviation of 1 6 μg per cent The 24 hour urinary excretion of free and glucuronic acid conjugated 17 OH C in 10 normal subjects varied from 8 to 21 mg per 24 hours The free 17 OH C component in this group was remarkably close to 10 per cent of the 24 hour excretion values given above

RESULTS

After adrenalectomy without any substitution therapy plasma levels of the free 17 OH C fell to zero in 4 of 7 patients However in only 1 of these 4 did the glucuronic acid conjugates in the plasma reach zero In the other 3 individuals of the 7 the free 17 OH C varied from 2 to 11 μg per cent and the glucuronic acid conjugates from 5 to 13 μg per cent In the 2 instances in which an infusion of ACTH was given after adrenalectomy the free 17 OH C in plasma rose in 1 and was unchanged in the other The data are presented in Table 1

In Table 2 are shown the plasma levels of 17 OH C and glucuronic acid conjugates in the plasma of 7 patients maintained after adrenalectomy on ammoniated glycyrrhizin alone Normal levels of these Porter Silber chromogens were obtained during glycyrrhizin substitution therapy and all were considerably higher than the range of values of the same materials when no replacement therapy was given

The 24 hour urinary excretion of the combined free and glucuronic acid conjugated 17 OH C were determined in 9 patients during a period of

Table 2 Plasma Levels 17 OH Corticosteroids Maintenance on Glycyrrhizin (The Values Given are in Micrograms %)

PATIENT	PREOPERATIVE FREE 17-OH CS	PREOPERATIVE GLUC ACID CONJUGATES	DAYS ON GLYCYRRHIZIN	FREE 17 OH CS	GLUC. ACID CONJUGATED 17 OH CS	CLINICAL RESPONSE TO ADRENALECTOMY
E T	—	—	8	2	19	Temporary subjective relief 2 mo then progression
P H	0	8	6	8	15	No improvement
O R	8	9	4	6	17	Too early to evaluate
E H	27	28	6	10	14	No improvement
F G	6	8	5	9.5	9.5	Excellent subjective and objective improvement 5 mo
W W	12	43	8	9	10	Good subjective and objective improvement 4 mo
K R	12	0	6	10	8	Too recent for evaluation

Table 1 Plasma Levels 17 OH Corticosteroids No Replacement Therapy

(The Values Given are in Micrograms %)

	PREOPERATIVE		TIME OF WITH DRAWAL	NO REPLACEMENT		ACTH		
PATIENT	FREE	GLUC ACID CONJUGATES	(HOURS)	FREE	GLUC ACID CONJUGATES	IV FREE	GLUC ACID CONJUGATES	CLINICAL RESPONSE TO ADRENALECTOMY
M T	28 5	14	68	11	13	16.5	0	No improvement death in 3 months
M D	9	22	96	0	13 5	0	5	Regression of all clinical evidence of disease 18 mo up to the present time
Z B	32	18	72	0	5	..		Subjective relief for 18 mo followed by rapid progression and death at 19 mo
L F	19	111	72	0	10	...	...	Subjective relief and change from bedridden to ambulation state for 20 mo then progression
E T		..	72	2	11			Temporary subjective relief 2 mo then progression
W W	12	43	72	0	0	..	..	Good subjective and objective improvement 4 mo
K R	12	0	72	4	11	...	...	Too recent for evaluation

along with the structural formula of hydrocortisone are shown for comparison in Figure 1 Glycyrrhizinic acid is formed by the combination of glycyrrhetinic acid and glucuronic acid[4] Ammoniated glycyrrhizin was administered 1 gm per day for from 7 to 18 days

The chemical methods used in the blood plasma determinations have recently been described in detail by one of us[6] The urinary free and glucuronic acid conjugates of the 17 OH C were similarly determined with appropriate modifications

Using the method referred to above[6] the plasma values for free 17 OH C in 42 normal human beings averaged 9 5 μg per cent with a standard

DISCUSSION

It is apparent that the numbers of observations reported have been too few to permit the formulation of definite conclusions regarding the possible explanation of causes of success or failure of adrenalectomy for carcinoma of the breast The finding of near normal concentrations of Porter Silber chromogens in the urine and plasma of adrenalectomized patients receiving only glycyrrhizin as substitution therapy was unexpected Hudson[7] observed no rise in the urinary 17 ketosteroid levels in adrenalectomized patients who were given glycyrrhizin However he did not exclude the possibility that in the metabolism of glycyrrhizin other steroids or non steroids capable of giving the Porter Silber reaction are produced To show that ammoniated glycyrrhizin does not itself contain a material giving the Porter Silber reaction the following experiment was performed To 2 of 4 100 ml portions of a patient s urine there were added 250 mg of ammoniated glycyrrhizin These 4 samples of urine were analyzed for free and 17 hydroxy conjugated corticosteroids Identical values were found in the 2 samples analyzed for free 17 OH C and in the 2 samples analyzed for conjugated 17 OH C This shows that the Porter Silber chromogens found in urine and plasma following ingestion of ammoniated glycyrrhizin were not present in the original drug but are probably metabolic products derived from the drug These products need not be steroidal since it is known that the Porter Silber reaction is only relatively specific for the 17 OH C and this lack of complete specificity may well be the explanation for our findings However by isolating the Porter Silber chromogen producing material from large quantities of urine from these patients we hope to be able by infra red spectrophotometry and other techniques to establish the chemical identity of these materials Another unexpected observation was the continuous excretion of considerable quantities of glucuronic acid conjugated 17 OH C and the maintenance of about half normal plasma levels in all but 2 patients given no replacement therapy after adrenalectomy Should the half life of cortisone be very long residual retention and excretion might explain this peculiar phenomenon However Hellman *et al*[8] have shown that 70 to 80 per cent of C^{14} labelled infused hydrocortisone was excreted in urine and feces in 24 hours and 90 per cent within 72 hours This makes the above possible explanation untenable

Another possible explanation for the maintenance of the above circulating plasma levels and urinary excretions is that these steroidal materials may be synthesized from cholesterol by gonadal tissue Since these patients were all castrates this possibility can be excluded

The eventual proof of the capacity of accessory adrenal tissue to secrete adrenal corticosteroids will be the isolation of such materials from accessory adrenal tissue removed at post mortem We are at the present time attempting to secure such tissue for analysis The experiments described above are also being continued it is our hope that more definite answers will be forthcoming soon

SUMMARY

1 The plasma levels of the 17 OH C have been studied in adrenalectomized patients in whom replacement therapy was withdrawn and following infusion of ACTH Evidence of incomplete adrenalectomy by the continued

Table 3 Urinary Excretion of Combined Free and Glucuronic Acid Conjugated 17 Hydroxycorticosteroids in Patients Maintained on Glycyrrhizin (The Values Given are in mg per 24 hours)

NAME	DAY			WITHDRAWAL OF CORTISONE THERAPY—DAYS																	
	3RD	2ND	1ST	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
I T		3 4	1 5		1 1	4 2		1 5	0 9	1 0	0 6	1 5	1 0	1 1	1 3	0 8	0 9	1 9	ACTH 1 6	0 2	0 3
W W			14 3		4 2	2 7	3 7	3 9	2 6	2 3	2 1	2 5	2 0	1 4	2 5	1 6	ACTH 1 5	2 2	ACTH 1 0	1 3	
P H			9 2			2 5	1 8	1 2	1 5	2 7	2 7										
K R				10 6		3 3	2 7	2 4	2 2	off 0 8	0 7										
A K					5 2	4 7	3 2	2 4	1 7	2 1	0 9	1 5	1 2	0 7							
E H			80	8 5	4 5		4 9	4 0	3 8	4 3	4 4	5 2	4 1	1 8	3 3	ACTH 4 0	3 7				
F G			9 4	8 2	6 8	3 0	3 6	3 5	ACTH 3 5	2 1											
O R	7 2	8 2	5 7	5 5	4 0	2 6	1 0	3 2	3 1	No Therapy 2 7	1 2	2 4									

withdrawal of cortisone and maintenance on glycyrrhizin In 4 patients ACTH (Gel) was given during the period of observation The period of study varied from 7 to 18 days The levels of 17 OH C on the sixth day of substitution therapy varied from 0 9 mg to 3 5 mg per 24 hours The giving of ACTH (Gel) in addition to the glycyrrhizin did not raise the urinary excretion of the 17 OH C over that obtained with glycyrrhizin alone

No correlation exists between these studies and the clinical results given in each table In the instance in which an increase of the plasma level of the free 17 OH C occurred following the administration of ACTH the clinical result was poor It is of interest that this patient was the only one who failed to develop symptoms of adrenal insufficiency after 96 hours without any replacement therapy In this case the single prolonged intra venous infusion of ACTH resulted in a rise in the plasma level of the free 17 OH C of 5 μg per cent It is unfortunate that in this case the urinary excretion was not determined However necropsy examination 3 months later did not uncover residual adrenal tissue or any accessory adrenal tissue However accessory tissue can be easily missed at necropsy Consequently the possibility of existence of accessory adrenal tissue in this case cannot be excluded with certainty

the sphenoidal air sinus and into the sella turcica Radon seeds are inserted and the cannula withdrawn

Twenty cases have now been treated The first patient had previously had adrenalectomy without benefit In the other 19 cases the pituitary implant has been done without previous endocrine treatment

The technique is not difficult and relatively trouble free We have had no deaths due to the implant One patient had a mild pyrexia lasting a few days Headache is variable usually slight and transient One patient early in the series developed a partial visual field defect due to damage to the chiasma by the cannula One other developed a field defect after 3 months probably due to malposition of one of the seeds too close to the chiasma

In this series we have had no dramatic response such as occasionally follows adrenalectomy but several cases have shown some evidence suggesting possible regression of the disease

Case 2 A woman aged 18 with skeletal metastases from breast cancer, was admitted with severe pain of spinal origin She was confined to bed and suffered intense pain on the slightest movement Radon seeds (10 mc.) were implanted February 16 1955 There was complete relief of pain within 2 days she got up from bed within a week and up to date is ambulant and pain free Serum calcium and alkaline phosphatase levels show evidence of skeletal recalcification

Case 3 A woman aged 49 with untreated breast cancer of 2 years duration having had no previous symptoms was admitted to the hospital with recurring Jacksonian convulsions originating in the right hand The convulsions deepened to *status epilepticus* At the seventh day radon seeds were implanted in the *sella turcica* The fits ceased within 2 days and have not recurred to date (6 months) We have no positive evidence of intracranial malignancy in this case and the improvement may have been coincidental

Case 9 A woman aged 50, with multiple skeletal metastases from breast cancer was submitted to pituitary radon implant on May 30 1955 X ray examination 3 months later showed marked recalcification of the skeleton with almost disappearance of some of the metastatic foci

Case 5 A woman aged 35 with intrathoracic deposits causing marked dyspnea has shown much symptomatic improvement

Case 8, with skin nodules and supraclavicular glands is thought to show some reduction in the size of the masses

Thus 5 patients out of 20 appear to have benefited Five others have died from progressive malignancy Some of the remainder have been done too recently to assess It is clear that in these early cases we have not destroyed the pituitary completely Autopsy in Case 1 at 28 days after 10 mc implant showed only one third of the gland necrotic though doubtless much more would have succumbed to late radiation damage if she had survived In another case 5 weeks after 15 *mc the destruction* was much greater but there was still a narrow rim of intact cells

Laboratory findings bear out this conclusion In some cases the 17 ketosteroids have been reduced to near zero in others they have remained unaffected F S H values have also been variable Polyuria has developed in some cases within a week or 2 of the implant In others it has developed gradually perhaps due to progressive radiation damage Electrolyte levels

presence of significant levels of 17 OH C and a rise in free 17 OH C following ACTH was noted in 1 case of 2 studied

2 The plasma levels and urinary excretion levels of the 17 OH C have been studied in 7 adrenalectomized patients maintained on ammoniated glycyrrhizin In this group of patients higher plasma levels of Porter Silber *chromogens were found in comparison with the first group of patients* maintained on no replacement therapy

In patients maintained on glycyrrhizin significant amounts of Porter Silber chromogens were excreted in the urine from 7 to 18 days following withdrawal of cortisone maintenance therapy The levels of urinary excretion of these materials was not elevated by the injection of ACTH (Gel)

3 The significance of these findings has been discussed

REFERENCES

1 Lasher E P Primary extrarenal hypernephroma discussion of sites and origins and report of a case West J Surg *55* 87 93 1947
2 Nelson A A Accessory adrenal cortical tissue A M A Arch Path *27* 955 965 1939
3 Graham L S Celiac accessory adrenal glands Cancer Phila *6* 149 152 1953
4 Ruzicka L and Leuenberger H Zur Kenntniss der Glycyrrhetinsaure Helvet chim acta *19* 1402 1936 Helvet chim acta *26* 2278 1943
5 Jenkins D Forsham P H Laidlaw J C Reddy W J Thorn G W Use of ACTH in the diagnosis of adrenal cortical insufficiency Amer J Med *18* 3 14 1955
6 Weichselbaum T E and Margraf H W Determination in plasma of free 17 hydroxy and 17-desoxy corticosteroids and their glucuronic acid conjugates J Clin Endocr Metab *15* 970 990 1955
7 Hudson P B Mittelman A and Mann P Urinary steroids after total adrenalectomy 17 KS in cancer patients maintained on cortisone and glycyrrhizin J Clin Endocr Metab *13* 1064 1069 1953
8 Hellman L Bradlow H L Adesman J Fukushima D K Kulp J L and Gallagher T F The fate of hydrocortisone-4 C^{14} in man J Clin Invest *33* 1106 1115 1954

A SIMPLE METHOD OF IMPLANTING RADON SEEDS INTO THE PITUITARY GLAND IN THE TREATMENT OF ADVANCED BREAST CANCER*

CHARLES F W ILLINGWORTH A P M FORREST AND D A PEEBLES BROWN

The value of adrenalectomy in hormone dependent breast cancer is well authenticated but the benefit is usually temporary The next logical step is hypophysectomy and encouraging results have been obtained[1] *As an alter*native to surgical hypophysectomy we have devised a method for destroying the pituitary gland by radon seeds.

The technique has been described by Forrest and Peebles Brown Under general anesthesia the patient is placed on a special head rest incorporating an adjustable director apparatus with radiopaque protractors in 2 planes A cannula is inserted through a director tube and along one nostril and with repeated x ray checks is advanced through the pharyngeal wall through

*From the University of Glasgow (Scotland)

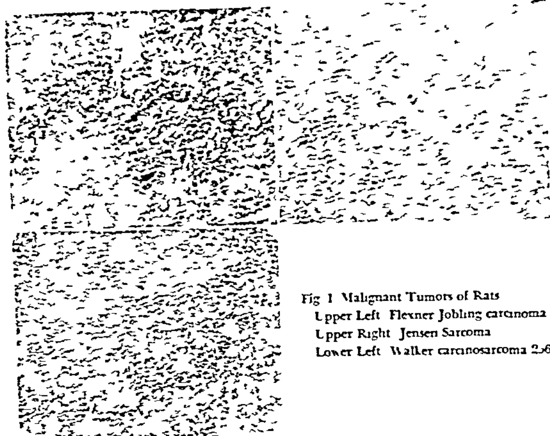


Fig 1 Malignant Tumors of Rats
Upper Left Flexner Jobling carcinoma
Upper Right Jensen Sarcoma
Lower Left Walker carcinosarcoma 256

malignant tumors namely Flexner Jobling carcinoma Jensen sarcoma and Walker carcinosarcoma 256* (Fig 1)

Forty-eight rats with tumor transplants that measured about 1 x 3 x 5 cm were used These rats were divided into 2 equal groups All the rats of the first group were cooled to 0 C. and maintained at this temperature for 2 hours The method of cooling and rewarming as described elsewhere[9] took an additional 2 to 3 hours All these rats were in cardiac standstill for at least 2½ hours during these experiments The second group of 24 rats were not cooled and they served as controls for the study

RESULTS

In the group of 24 rats which were cooled to 0 C. 4 died during rewarming and 4 more survived the cooling but died 1 to 7 days afterward These rats were excluded from this study as their survival was too short for any evaluation The remaining 16 rats which survived cooling to 0 C. lived for periods of several months Four of these 16 rats with long survival showed a steady increase in the size of their tumors which finally killed them The remaining 12 rats showed a gradual regression of their tumors and in 10 of them the tumors were replaced by small nodules which measured 3 to 4 mm in diameter The nodules were abscess cavities (Fig 2)

The regression in the size of the tumor was first noticed 2 weeks after cooling During this interval the growth of both the animal and the tumor was temporarily retarded as compared to their control mates After this

*We gratefully acknowledge Dr Kanematsu Sugiura Sloan Kettering Institute New York Dr G A LePage McArdle Memorial Laboratory Wisconsin and Dr P A Herbut Jefferson Medical College Philadelphia Pennsylvania for supplying us with these tumors.

4 Pack C T and Adair F F Subungual melanoma Surgery 5 47 1939
5 Pack C T Symposium on tumors of the hands and feet Introduction Surgery 5 1 1939
6 Geschickter C F and Copeland M M Tumors of bone Ed 2 New York Am J Cancer 1936 p 491
7 Fay T and Henry C C Correlation of body segmental temperature and its relation to the location of carcinomatous metastasis Clinical observations and response to methods of refrigeration Surg Gyn Obst 66 512 1938
8 Smith L W and Fay T Temperature factors in cancer and embryonal cell growth J Am M Ass 113 653 1939
9 Niazi S A and Lewis F J Tolerance of adult rats to profound hypothermia and simultaneous cardiac standstill Surgery 36 25 1954
10 Treloar A E Biometric Analysis Minneapolis Burgess Publications Co 1951
11 Earle W R A study of the Walker rat mammary carcinoma 256 in vivo and in vitro Am J Cancer 24 566 1935
12 Schrek R A quantitative study of the growth of the walker rat tumor and the Flexner Jobling rat carcinoma Am J Cancer 24 807 1935
13 Jensen C O Übertragbare Rattensarkome Zschr Kreislaufforsch 7 45 1908

URINE CALCIUM EXCRETION STUDIES AS A MEANS OF SELECTION OF HORMONE DEPENDENT TUMORS*

ANDREW G JESSIMAN

The treatment of mammary and prostatic cancer has undergone many changes in its evolution since it was first appreciated in the late nineteenth century[1] that there was an interrelationship between the gonads and these neoplasms

Throughout this period the role of surgery in carcinoma of the breast has varied from the extreme ultra radical mastectomy[3] to the present view shared by most workers in this field that radical mastectomy should be reserved for a very limited number of cases—Stage I All the other more advanced cases are treated either with x ray therapy or by an hormonal attack on the disease Alteration of the hormonal environment of the cancer cell either by the administration of synthetic sex hormones or by the ablation of the glands producing or controlling the excretion of these steroids may give considerable remissions[4 5 6 7]

Numerous reports of this hormonal treatment indicate that only about 30 per cent mammary cancers and 80 per cent prostatic cancers are being stimulated by or are dependent upon the level of the appropriate circulating sex steroid

In recent years in this rapidly expanding field cortisone suppression of the adrenal cortex has been added to the available hormones and in the surgical field the operations of adrenalectomy and hypophysectomy are giving gratifying results These operations carry with them a surgical mortality and an operative morbidity and it is obvious that the correct selection of those cases that will respond to these procedures is of critical importance

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Fig 2 Specimen taken one month after cooling showing pus formation surrounded by an abscess wall

period the animal resumed its usual growth while the tumor continued to regress

In the control group of 24 rats only 4 rats showed spontaneous regression while the tumors of the remaining 20 rats continued to grow until they killed their hosts The difference between the results obtained in the controls and the treated groups appears to be significant The probability that this difference would occur due to the errors of random sampling is less than 001 according to the Chi squared test[10]

DISCUSSION

These 3 kinds of tumors were evenly distributed among the groups of rats studied Spontaneous regression has been rarely reported in the Walker carcinosarcoma 256[11 12] Eighteen and 25 per cent regression has been reported in the Jensen sarcoma and Flexner Jobling carcinoma respectively[13 1] In our study spontaneous regression occurred in 4 rats of the control group of 24 rats (16 7 per cent) whereas hypothermia caused regression in 10 rats of the group of 16 rats which had long survival after cooling to 0°C (62 5 per cent) This effect of profound hypothermia on the transplanted malignant tumors should be further evaluated in animals with spontaneous malignant tumors because we do not know yet whether the effect of hypothermia on the malignant cell is a direct one or it is mediated through the host carrying the tumor

SUMMARY

1 Sprague Dawley rats were transplanted with 3 kinds of malignant tumors Flexner Jobling carcinoma Jensen sarcoma and Walker Carcinosarcoma 256

2 Cooling to 0 C produced regression in 10 rats out of 16 with tumor transplants

3 Spontaneous regression of these tumors occurred in 4 rats of the control group of 24 rats

REFERENCES

1 Huggins C and Noonan W J An increase in reticulo endothelial cells in outlying bone marrow consequent upon a local increase in temperature J Exper M 54 2/5 1936

2 Coley B L and Higinbotham N L Tumors primary in the bones of the hands and feet Surgery 5 112 1939

3 Mason M L Carcinoma of the hands and feet Surgery 5 27 1939

It is obvious that if there are only a very few osseous metastases however dependent the tumor may be and active the metastases become they can not produce sufficient osteolysis to alter the daily calcium excretion level The same will apply if there are numerous osseous metastases and the tumor is only of moderate hormone dependency (b) the systemic response of the patient to this stimulating dose Increase in bone pain malaise nausea pyrexia and even mental confusion or coma indicate a positive response and therefore hormone dependency of the tumor

When a stimulation test is being carried out careful daily appraisal of the patient s condition must be made for hypercalcemia and renal failure can occur and be dangerous To avoid these complications the test should not be done

In the presence of pre-existing hypercalcemia

When on a limited daily 200 mg intake the daily urinary calcium excretion is more than 300 mg

In the presence of decreased renal function

Inhibition Test Those patients who following gonadectomy show progression of the disease may have a high level of circulating sex hormones of adrenal origin If their tumors are hormone dependent a further clinical remission may be achieved by adrenalectomy and hypophysectomy The selection of these cases may be made by observing the urine calcium excretion when the adrenal cortex is suppressed with cortisone Adequate cortical suppression is observed by studying the urinary 17 hydroxycorticos teroids and 17 ketosteroid levels and noting a good fall

A fall in the urine calcium excretion indicates that the tumor is hormone dependent and that an adrenalectomy or hypophysectomy will be beneficial and conversely an unchanged level intimates lack of dependency It is this latter group that can be spared the mortality and morbidity of further unnecessary surgical attacks on the endocrine system

SUMMARY

1 There is a need for adequate selection of those patients with mammary or prostatic cancer who have hormone dependent tumors

2 A study of the daily calcium excretion in a patient on a standard calcium intake can be used as an index of the natural rate of growth of the disease

3 A stimulation test is described in which the result is judged in 2 moieties—by the change in urine calcium excretion and by the systemic response of the patient

4 The adrenal inhibition test can be used to select those patients who having had a gonadectomy are likely to benefit from adrenalectomy or hypophysectomy

5 It is hoped that these tests will lead to a more rational approach to therapy in the handling of those patients with advanced malignant disease of the breast and prostate glands

REFERENCES

1 Beatson G T On the treatment of inoperable cancer of the mamma Suggestion for a new method of treatment with illustrative cases Lancet Lond 2 104 1896

2 Boyd I On oophorectomy in cancer of the breast Brit M J 2 1161 1900

Previous attempts at selection have been based on the response of the patient to gonadectomy or hormone therapy. Those patients responding well to these measures would benefit from a further attack on the hormonal field by adrenalectomy or hypophysectomy.

There would appear to be little evidence to support the belief held by some workers that the histological type of the primary tumor can be used as a guide to its hormone dependency.[8]

Pearson[9,10,11] in his study of advanced prostatic and mammary cancer was the first to appreciate and demonstrate that a study of the daily urine calcium excretion in patients with osseous metastases could be used as an index of the rate of progress of the disease. Metastases will replace the bone matrix volume for volume and it can be calculated that when 1 gm. of bone is replaced by neoplastic tissue, approximately 100 mg. calcium are excreted in the urine. Stimulation or inhibition of the tumor will be reflected in its rate of growth and a corresponding change in the daily urine calcium excretion.

By following the trend of the urinary calcium excretion levels the rate of natural growth of the tumor can be measured. As well as this the hormone dependency of a tumor may be assessed by its stimulation or inhibition by the appropriate sex steroid.

METHOD

The patient is placed on a diet containing a maximum daily intake of 200 mg. calcium and after 3 days on this regime 24 hourly urine collections are started and the urine calcium determined. A normal patient on this regime will excrete 50 to 100 mg. calcium a day but a patient with osseous metastases may excrete up to 1000 mg. calcium or more in the same period.

Observations of the Daily Level of Urinary Calcium Excretion. A raised daily level of 100 mg. or more above the average daily level indicates activity of the disease and growth of the osseous metastases. Multiple short term observations lasting a few days and made at intervals of a few months will give a picture of the progress of the disease, a rising level at each successive evaluation indicating increasing activity and a need for a further therapeutic change.

Furthermore, observations of the change in urine calcium excretion following gonadectomy will give a good indication of the dependency of the tumor and this when combined with the clinical response can be used in predicting the success or failure of any further therapy that may be contemplated such as adrenalectomy or hypophysectomy.

Stimulation Test. The administration of a stimulating dose of 150 mg. testosterone propionate or 10 mg. stilbesterol per day to carcinoma of the prostate or breast respectively for 3 days will activate a hormone dependent tumor.

Assessment of the result is made by (a) following the daily urine calcium excretion. In a dependent tumor the growth of the osseous metastases causes a rise in the daily excretion levels. This rise may be delayed until a few days after the administration of the test dose as the mobilization of calcium from the bones occurs slowly. However once it is seen it may last from 2 to 4 weeks. The extent and duration of this rise will depend on both the number of osseous deposits present and also on the degree of dependency of the tumor for all tumors are not equally dependent.

cancer patient may induce temporary hot flashes and amelioration of symptoms for 1 to 3 weeks, but not infrequently is then followed by rapid relapse with cessation of hot flashes This relapse is probably due to the heightened requirements for adrenal corticoids following operative stress Cortisone administration begun when the patient relapses after oophorectomy often induces a remission of some months duration Hot flashes reappear within 1 to 6 weeks of commencement of endocrine therapy in these patients In patients older than 65 to 70 years preliminary surgical castration may *not be necessary* since cortisone therapy may be highly effective and has induced remissions of 12 to 21 months in duration in patients with intact ovaries Postmenopausal noncastrate patients do not manifest hot flashes, however at any time if they have been free of estrogen withdrawal symptoms until initiation of treatment It should be emphasized that radiation therapy is unreliable for destruction of ovarian estrogen production to the extent necessary for successful cortisone therapy

Cortisone and Thyroid Dosage Cortisone therapy has been initiated with a 200 to 300 mg dose the first day followed by 100 to 150 mg daily for the first week No direct pathway of metabolism of cortisone to estrogen has been discovered Munson and co workers have shown that cortisone in doses exceeding 100 mg daily is converted to androgenic steroids[6] which may be transformed secondarily to estrogenic substances by tissue metabolism[7] The effectiveness of cortisone in suppression of endogenous estrogen production therefore depends upon maintaining therapy after the first week or two with doses of 100 mg daily or less The hormone should be administered in divided doses throughout the 21 hours to produce maximal sustained adrenocortical suppression and in some patients with poor absorption parenteral maintenance therapy may be necessary, preferably with 50 to 75 mg total daily dose Hydrocortisone appears to be equally as effective as cortisone therapy

Dessicated thyroid in 60 to 120 mg doses has been added after some weeks of cortisone medication Restriction of dietary salt has seldom been necessary but 2 to 1 gm potassium chloride has been routinely prescribed with the cortisone Some patients have been given supplementary vitamin B12 50 mcg orally daily and 30 mcg intramuscularly each week No sex hormone should be administered concurrently with cortisone In 2 patients with the longest remissions of 18 and 21 months x ray therapy to areas of bone destruction was necessary during the first 3 months of cortisone In 2 patients with massive hepatic metastases intravenous HN 0 1 to 0 6 mg /kg total dose was given concurrently with cortisone and thyroid

Indices of Remission Functional evaluation of the patient has included serial estimation of body weight in edema free cases estimation of the appetite and amount of physical activity possible without pain Changes in size of metastases have been recorded photographically whenever possible Early laboratory manifestations of remission have been obtained from serial urinary calcium estimations with the patient on a low calcium diet and serial observations of copper resistant serum acid phenylphosphatase using the method developed by Dr M D Reynolds in our laboratory[8] Serial observations have been completed in a few cases of urinary follicle stimulating hormone (immature mouse uterus method) and of androsterone and etiocholanolone glucuronides

3 Urban J A and Baker H W Radical mastectomy in continuity with en bloc resection of the internal mammary lymph node chain Cancer Phila 5 992 1952
4 Nathanson I T Influence of stilbesterol on advanced cancer of the breast J Clin Invest 25 930 1946
5 Haddow A Watkinson J M Paterson F and Koller I C Influence of synthetic estrogen upon advanced malignant disease Brit M J 2 393 1944
6 Cade Sir S Adrenalectomy for breast cancer Brit M J 1 1 1955
7 Harrison J H Thorn G W and Jenkins D Total adrenalectomy for reactivated carcinoma of the prostate N England J M 248 86 1953
8 Huggins C Endocrine methods of treatment of cancer of the breast J Nat Cancer Inst 15 1 1954
9 Pearson O H West C D and Treves N The role of ovarian function in the growth of mammary cancer in man J Clin Invest 32 591 1953
10 Pearson O H and West C D Objective methods for the measurement of tumor growth in man Proc Am Ass Cancer Res 1 42 1953
11 Pearson O H West C D Hollander V P and Fscher G C Alteration in calcium metabolism in patients with osteolytic tumors J Clin Endocr Metab 12 926 1952

ARREST OF METASTATIC MAMMARY CARCINOMA BY CORTISONE AND THYROID THERAPY*

HENRY M LEMON

Castration total adrenalectomy and total hypophysectomy have aided in the treatment of metastatic breast cancer indicating that in some patients endocrine cancer stimuli originate from the removed organs Cortisone substitution therapy is necessary however for optimum maintenance of adrenalectomized and hypophysectomized patients and benefits observed postoperatively may be due in part to the nature of the medical therapy required Since the first observations of arrest of mammary cancer metastases by cortisone therapy alone[1,2] numerous additional patients have been treated The subject of this report concerns nearly 15 patient years experience with cortisone and thyroid therapy of 30 unselected cases in all stages of cancer metastasis About one half of these have had objectively measurable remissions and in one quarter remissions have lasted in excess of 6 months

METHOD

Castration Female sex hormones are produced in the adrenal cortex as well as in the gonads Although cortisone administration suppresses pituitary ACTH secretion it tends to enhance follicle stimulating hormone secretion and excretion[3] Some previous investigators have neglected to castrate patients prior to cortisone administration which we have found is a necessary precaution up to the age of 65 since there is biochemical and pathologic evidence of active ovarian secretory activity well past menopause[4,5] Castration of the menopausal or post menopausal breast

*These studies have been carried out with the aid of teaching and research grants from the National Cancer Institute National Institutes of Health U S Department of Health Education and Welfare an institutional cancer research grant to Boston University from the American Cancer Society and research grants from the Massachusetts Division of the American Cancer Society Merck Inc generously provided steroids used therapeutically

Table 1 Regressions Noted in Patients Receiving Therapy for One Month or Longer

	Areas of Metastasis											
	Brain	Lung & Pleura	Mediastinum	Liver	Skin	Periph Ln	Skull	Spine	Pelvis	Femurs	Other Skel	Other Soft
Responding to steroid (objective improvement in function)	0	3	0	2	3	3	2	6	4	2	2	0
Total Cases	1	6	2	6	6	5	3	8	5	4	4	2

Table 2 Survival of Patients on Cortisone Thyroid Therapy

Duration Therapy	No Pts	No Living	*Survivors Health*
1 2 years	5	2	1 well 1 terminal after 12 mo remission
6 12 mos	4	1	In remission gainfully employed
3 6 mos	9	5	1 well 1 improved 3 progressive dis
1 3 mos	8	2	2 unimproved therapy discontinued
Duration Remissions			
1 2 years	4		
6 12 mos	4		
3 6 mos	6		
1 3 mos	3		

phatase activity again tended to rise to a mean preterminal value of 19 0 $\mu M/100$ ml

Urinary Calcium Excretion Serial observations disclosed rapid reduction of elevated urinary calcium within 48 hours after commencing cortisone in 1 patient with extensive bone involvement who ultimately showed marked bone repair in addition to recalcification and who is still well after 21 months A second similar patient with myxedema failed to show any decrease in urinary calcium until 15 mg of thyroid was initiated and subsequently showed evidence of control of osseous metastases A third patient receiving cortisone thyroid and local x ray for osseous metastases continued to excrete large amounts of calcium during the initial weeks of treatment and proved to have progressive bone disease during the next 4 months Elevated urine calcium output has been noted in bed ridden patients without detectable osseous metastases which did not respond to cortisone therapy

Duration of Remission and Patient Survival Effective anti tumor treatment should increase survival time of patients with metastatic cancer In Table 2 is presented the distribution of objective remissions according to their duration the accompanying length of survival of patients and their present condition if alive (in remission or in relapse) In patients who have relapsed estrogen or androgen therapy and total adrenalectomy have failed to be beneficial

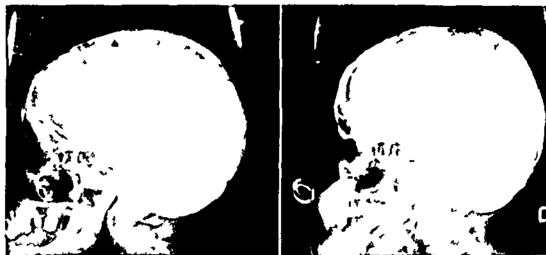


Fig 1 Repair of Cranial Metastases—Mrs M S Age 70 Left before treatment December 9 1953 Right after 17 months on 75 to 50 mg cortisone and 15 mg thyroid daily June 8 1955

RESULTS

Pain Relief A striking and immediate (within 18 hours) relief of pain has occurred in patients with osteolytic metastases after receiving 300 mg of cortisone Such a dramatic initial response usually was followed *by a sustained remission lasting some months In 1 patient who received* testosterone concurrently pain relief was not marked for some weeks until some time had elapsed since her last testosterone injection In several patients pain relief was complete requiring no further analgesics

Ambulation Most patients were semi ambulatory at the start of treatment but several were bed ridden by osseous involvement In nearly all those who improved and are classed as remissions exercise tolerance improved until light housework was possible Cortisone in several patients permitted continued ambulation during local x ray therapy of osseous or soft tissue involvement In only 1 instance did bone metastases prevent ambulation and this patient did not benefit from treatment

Weight Gains Ambulatory patients in remission gained from 10 to 35 pounds in weight without clinical edema after 3 to 21 months of therapy A marked improvement in appetite and other objective evidence of remission was noted in all these patients

Response of Metastases to Therapy Regressions of soft tissue and osseous metastases have been noted in patients in all age groups as shown in Table 1 Only central nervous system lesions have thus far been disappointing in their therapeutic response No generalizations are yet possible from this small experience in view of the numerous factors concerned in growth of metastases

Copper resistant Serum Acid Phenylphosphatase Untreated patients with metastatic disease showed a mean serum activity of 32 8 ± 5 6 μM phenol/100 ml serum compared to 11 3 ± 0 8 in volunteers and 11 8 ± 0 8 in patients with benign diseases After 1 week to 1 year of treatment all except 2 patients of a series of 16 showed a decline in this enzyme to a mean value of 20 8 μM/100 ml within the range of normal which does not exceed 21 5 μM/100 ml Subsequently as disease reactivated acid phos

A PLANE INTERSTITIAL ISOTOPE APPLICATOR FOR IRRADIATION OF THE MEDIASTINUM*

P. V. Harper, William L. Adams, Emanuel I. Schwartz,
Katherine A. Lathrop and Robert W. Harrison

Although it is usually impossible for a patient to tolerate cancericidal radiation dosage to the mediastinum from external irradiation, it is possible with a plane gamma ray source applied to the mediastinum to reach very high superficial dose levels. The rapid fall off of the radiation field surrounding such an applicator reduces the radiation to the vulnerable trachea, esophagus and lung to tolerable levels. The technical details of such an approach have been worked out in experimental animals and form the basis for this report.

METHOD

Applicators were constructed using blotting paper of appropriate dimensions sealed in envelopes of polyethylene sheeting. These were implanted in experimental animals against the mediastinum (Fig. 1). Several days later the blotting paper was saturated with a solution of a suitable isotope introduced through fine polyethylene tubing sealed into the envelope.

Envelopes constructed of .002" polyethylene sheeting were satisfactory but had to be handled very delicately to avoid perforations and leaks and became easily distorted by contraction of scar tissue. Envelopes made with .01" sheeting were substantial but somewhat less pliable to handle. Very fine polyethylene tubing (Clay Adams PL 10) was sealed into the envelope



Fig. 1. Roentgenogram showing 3x15 cm. applicator in the chest of an experimental animal. The blotting paper has been saturated with 3 cc. of saturated KI solution containing 45 mc. of I^{131} giving a dose of 5000 r 1 cm. from the applicator. Sievert chambers are present in the esophagus.

*From the Argonne Cancer Research Hospital and The Department of Surgery, University of Chicago Clinics, Chicago, Illinois. This study was aided by grants from the Damon Runyon Memorial Fund and The Douglas Smith Foundation for Medical Research of the University of Chicago.

Side effects. Manifestations of hypercortisonism have been severe in only 2 patients aged 27 and 35 years in whom large doses were initially required for palliation. One patient had a terminal gastric hemorrhage after 4 months therapy. No hypertension or persistent edema occurred with these small maintenance doses. Two patients developed symptoms of adrenal insufficiency, 1 following a severe pulmonary infection, and the other after cortisone withdrawal, resulting in fatal coma.

Effect of Therapy on Endocrine Function. During treatment urinary follicle stimulating hormone increased from low (6 m.u.) or normal levels (26 m.u.) to high values (106 m.u.) in several patients or was maintained at a high level indicative of decreased pituitary inhibition by circulating estrogen. Treated patients excreted one-tenth the usual amount of glucuronide conjugated androsterone and etiocholanelone compared to untreated patients and normals, indicating marked suppression of adrenal sex hormone production.

CONCLUSIONS

Cortisone in 75 to 100 mgm. daily maintenance doses is an effective palliative agent in about one-half of patients with metastatic mammary cancer. Thyroid supplementation in 60 to 120 mg. daily doses appears to act synergistically with cortisone since one-quarter of our patients have had remission in excess of 6 months in contrast to 3 month remissions reported for cortisone alone.[2] Ovarian function must be absent as a result of senescence (age over 65 to 70 years) or surgical castration for cortisone treatment to be effective. Serial urinary follicle stimulating hormone and androsterone excretion studies in treated patients are consistent with a marked suppression of endogenous adrenocortical sex hormone production.

REFERENCES

1. Lemon, H. M., and Davison, M. M.: Arrest of mammary carcinoma by cortisone and hydrocortisone. Clin. Res. Proc., *2*: 100, 1954.
2. West, C. D., Li, M. C., MacLean, J. P., Escher, G. C., and Pearson, O. H.: Cortisone induced remissions in women with metastatic mammary cancer. Proc. Am. Ass. Cancer Res., *1*: 51-2, 1954.
3. Sohval, A. R., and Soffer, L. J.: The influence of cortisone and adrenocorticotropin on urinary gonadotropin excretion. J. Clin. Endocr. Metab., *11*: 677-87, 1951.
4. Sommers, S. C.: Endocrine abnormalities in women with breast cancer. Laborat. Invest., *4*: 160-74, 1955.
5. Smith, O. W., and Emerson, K.: Urinary estrogens and related compounds in post-menopausal women with mammary cancer: effect of cortisone treatment. Proc. Soc. Exp. Biol., N. Y., *85*: 264-7, 1954.
6. Munson, P. L., Goetz, F. C., Laidlaw, J. C., Harrison, J. H., and Thorn, G. W.: Effect of adrenocortical steroids on androgen excretion by adrenalectomized orchidectomized men. J. Clin. Endocr. Metab., *14*: 495-508, 1954.
7. Baggett, B., and Engel, L. L., Savard, K., and Dorfman, R. I.: Formation of estradiol-17B-C^{14} from testosterone-3-C^{14} by surviving human ovarian slices. Fed. Proc., Balt., *14*: 175-6, 1955.
8. Reynolds, M. D. D., Lemon, H. M., and Byrnes, W. W.: Copper resistant serum acid phenylphosphatase in patients with benign and neoplastic disease. Proc. Am. Ass. Cancer Res., *2*: 41, 1955.

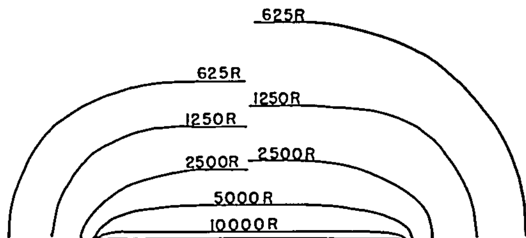


Fig 2 Isodose curves in vertical plane through center of 10x10 cm applicator Isotope density for I^{131} is 70 mc/cm² for Cs^{131} is 3 6 mc./cm² Dosage is calculated for complete decay of isotope





Fig 3 Effect of applicator on adjacent lung tissue 6 weeks after isotope administration Isotope density of I 1 0 mc/cm (a) Cs^{131} 1 60 mc/cm (b) Note dense scar tissue adjacent to relatively normal lung tissue In the I^{131} implant (a) the beta ray field has destroyed the cellular elements in the scar tissue

for subsequent introduction of the isotope, and the applicator was tested for leaks by evacuating it and submerging it in zephiran solution overnight. No further sterilization was used. Various methods of fixing the envelopes in place were found satisfactory. Wide borders were left on the envelope through which sutures were passed. Perforations in the borders made this process easier and sutures were sealed directly into the borders of the envelope.

The end of the polyethylene tubing was brought out through a stab wound and left in a small coil under the skin. When it was desired to introduce the isotope the end of the tubing was exposed under local anesthesia, the isotope solution was injected from a shielded syringe in a sufficient volume to saturate the blotting paper evenly and a sufficient isotope density to give the desired dosage. The tubing was cut off, sealed and left beneath the skin. It was found that an isotope solution containing substantial amount of carrier could be removed at any time to any desired extent by repeated washing of the envelope via the tubing. With reasonable care radiation and isotopic contamination were negligible.

The effects of two isotopes were investigated. The first was I^{131} because of its availability and its suitable half life and gamma radiation. The second isotope Cs^{131} has a half life of 10 days.[1] It was chosen because it decays by K capture consequently emitting the soft characteristic x rays of Xenon (29 kv). The range of this radiation in tissue is much less than that of the hard gamma radiation, the true absorption being about 17 per cent per cm as compared to 3 per cent per cm for I^{131} and radium gamma rays. Dose measurements were made surrounding point sources of these isotopes in a water phantom using a small ion chamber. From these data using methods of graphical integration isodose surfaces were calculated surrounding applicators of various sizes and shapes (Fig. 2).

RESULTS

In 8 animals 3x15 cm applicators were placed against the mediastinum (Fig. 1). They were well tolerated and none became infected. There was no hematologic depression in any of the animals using a radiation dosage of 5000 r at 1 cm from the center of the applicator. In 1 animal with an I^{131} implant Sievert chamber measurements in the esophagus confirmed the calculated radiation dosage within the limits of error.

In considering dosimetry it was found that within 3 cm of the I^{131} source in the water phantom, the region with which we are most concerned in implant work, scattered radiation compensated for absorption almost completely as is true with the radiation of radium and cobalt^{60}.[2] *The I^{131} radiation field resembles closely that produced by radium.* In the Cs^{131} field close to the source scattered radiation increased the dose rate about 20 per cent above the air dose. The gradient of the field was quite steep due to absorption in the water. Fig. 2 shows the isodose curves in a vertical plane through the center of a 10x10 cm applicator. Immediately adjacent to the applicator the radiation field of both isotopes is extremely high producing essentially a local cautery of the tissue. Fig. 3 shows the effects of this on adjacent lung tissue. At a distance of 10 cm from the applicator on the central axis of the applicator the Cs^{131} field falls to one fifth of the I^{131} field.

Clinical and Experimental Studies on Organ Transplantation

SECONDARY KIDNEY HOMOTRANSPLANTATION*

RICHARD HARRISON EGDAHL AND DAVID M. HUME

The mechanism of homograft destruction remains unexplained. However, there is a considerable amount of indirect evidence that the homograft reaction is determined by an immune mechanism, the most convincing example of which is the so-called "second set" phenomenon.

Medawar[1] has demonstrated that primary skin homografts in rabbits have a median survival time of 15.6 days, whereas second grafts from the same donor to the same host have a median survival time of 6.0 days. Dempster has stated that secondary kidney homotransplants from the same donor function and survive for a shorter interval than the primary.[2,3] However, there are three objections to these experiments: (1) the same site for both primary and secondary kidney homotransplantations was used, opening the possibility that a local concentration of antibodies had been left around the first graft; (2) crossed transplants were performed in most instances so that the donor's antibody mechanism may have been altered before his second kidney was removed; and (3) in some experiments functional periods were not determined and the transplants were arbitrarily removed at 4 days.[4,5]

The present study consists of a series of 60 primary and 15 secondary renal homotransplants performed in dogs, with a comparison of pathological findings and secretory intervals. The secondary transplants were performed at a site remote from the primary to rule out local antibody concentration. The interval between primary and secondary homotransplants was varied in different experiments to determine the length of "the immune state" in the host animal.

METHOD

Healthy adult mongrel dogs ranging in weight from 12 to 25 kg were used in the study. Intravenous sodium pentobarbital was the anesthetic agent and all operations were performed under aseptic conditions. Penicillin, 300,000 units intramuscularly, was administered on the 2 days following operative procedures. For each transplantation experiment, dogs close in weight but very dissimilar in body characteristics were selected.

Through a right subcostal incision the donor kidney was prepared for

* From the Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland.

DISCUSSION

It appears that the present method could be used clinically to deliver intense radiation to large areas in the mediastinum to a depth of a centimeter or so without producing serious reaction in the patient and without radiation hazard to the surgeon Applicators of suitable sizes could be kept sterile and available for use in suitable cases at any time Isotope administration could be carried out at leisure, after the patient recovered and after a bronchial stump had time to heal etc The patient may even be sent to another institution for isotope administration if necessary The use of Cs^{131} although it is not at present available shows great promise of allowing more extensive implants than can be tolerated using ordinary radiation because of the much more marked localization of the radiation field

It is felt that a sufficient number of cases of carcinoma of the lung might have small superficial mediastinal metastases accessible to this type of radiation field to make its prophylactic use following pneumonectomy worthy of clinical trial

SUMMARY

A general method is presented for using isotopes in solution in plane interstitial applicators Dosimetric consideration for I^{131} and Cs^{131} are discussed and experimental observations of such applicators in the mediastinum have been made in dogs The method appears practical from a clinical standpoint

REFERENCES

1 Harper P V and K A Lathrop The feasibility of using Cs^{131} K radiation in therapy Semi annual report to the AEC ACRH 2 L O Jacobson Editor 62 68 Sept 1954
2 P Wootton R J Shalek and G H Fletcher Investigation of the effective absorption of radium and $cobalt^{60}$ gamma radiation in water and its clinical significance Am J Roentg 71 683 690 1954

Table 1 Functional Contrast Between Primary and Secondary Kidney Homotransplants

EXPT NO	DAYS OF FUNCTION* PRIMARY	SECONDARY	DAYS BETWEEN TRANSPLANTS	SITE OF PRIMARY	SITE OF SECONDARY
1	3	3	37	Renal Fossa	Neck
2	9	1	14	Renal Fossa	Neck
3		1	18	Renal Fossa	Neck
4	7	2	133	Renal Fossa	Neck
	[illegible]	0	29	Renal Fossa	Neck
6	4	3	27	Neck	Renal Fossa
7	3	0	[illegible]	Neck	Renal Fossa
8	4	0	23	Neck	Renal Fossa
9	6	2	17	Renal Fossa	Neck
10	10	1	41	Renal Fossa	Neck
11	6	1	14	Renal Fossa	Neck
12	14	5	21	Renal Fossa	Neck
13	3	1	21	Renal Fossa	Neck
14	7	2+	73	Renal Fossa	Neck
15	7	1	17	Renal Fossa	Neck

*A day of function implies a urinary output by the transplant of 50 cc. or more

the primary homotransplantation reaction is interstitial round cell infiltration with variable amounts of interstitial edema and hemorrhage cortical ischemia tubular necrosis and necrotizing arteritis of small blood vessels with endothelial proliferation and obstruction of the lumen with cells and clot One transplant secreted over 100 cc of urine of specific gravity around 1 010 for several days and many transplants secreted large quantities of urine of specific gravity between 1 020 and 1 030 Efforts were made to keep the animals well hydrated and of course very large urine outputs were usually of low specific gravity As a general rule albumin in the urine increased and gross hematuria appeared near the end of the functional periods

Contrast Between Primary and Secondary Homotransplantations Table 1 indicates the secretory intervals for both primary and secondary homotransplantations in the same pair of dogs It can be seen that in only 1 experiment did *the secondary transplant function* for as long a time as did the pirmary and 9 of the 15 secondary transplants functioned for 1 day or less One secondary homotransplant functioned for 5 days but this is in contrast to a 14 day secretory period in the primary Varying the time interval between transplantations did not seem to affect the relatively shorter secretory interval of the secondary homotransplant for a secondary performed 133 days after the primary only functioned for 2 days compared to 7 days for the primary

The marked contrast between primaries and secondaries was illustrated by the pathological findings Grossly the primary homotransplant is much enlarged and edematous and the secondary is slightly enlarged and intensely hemorrhagic Interstitial round cell proliferation and necrotizing

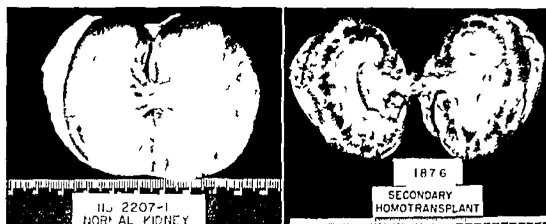


Fig 1 Normal dog kidney

Fig 2 Secondary kidney homotransplant removed after 1 day of function Note hemorrhagic areas in both cortex and medulla as well as darkness of cut surface generally (microscopic hemorrhage)

transplantation[6] A recipient site was made ready either by removing the right kidney or by exposing the carotid artery and external jugular vein in the neck Suture anastomosis of both vein and artery was performed with 5 0 silk and renal anoxia time for the neck transplants was approximately 15 minutes as compared to a time of 20 to 25 minutes for those kidneys transplanted in the renal fossa The donor kidney for the primary homotransplantations was usually taken from the left side because there is a double renal artery on the left in approximately 50 per cent of dogs The ureter of the transplant was cannulated with a #190 polyethylene tubing (Clay Adams animal tested) which was brought out through a stab wound and sutured to the side of the animal The polyethylene catheter was connected to a plastic bag which was pinned to a canvas jacket placed on the animal to prevent him from disturbing the bag or catheter

Daily collections of urine were made by emptying the contents of the plastic bag and specific gravity albumen urea creatinine and microscopic examinations were performed When urine volumes fell below 50 cc per day or blood appeared in the ureteral catheter the transplantation site was explored Blood flow through the transplant was roughly determined by clamping the renal vein distally and cutting it proximally The renal vein and artery of the transplant were carefully dissected after its removal the cut surface of the kidney was closely inspected and cross sectional pieces of the kidney were placed in 10 per cent formalin for histological preparation and microscopic examination

RESULTS

Summary of Findings in 60 Primary Kidney Homotransplants Fifty six of the 60 primary kidney homotransplants secreted urine with an average secretory period of 5 9 days Of the remaining 4 transplants 3 were technical failures and 1 was an example of the anuric kidney as it has been called by Dempster The technical difficulties occurred early in the course of our experiences and the last 30 transplants were performed uneventfully

Grossly, the primary transplants were much enlarged with diffuse mottling and edema of the cut surface The principle microscopic feature of

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3	5	1	18	Renal Fossa	Neck
4	7	2	133	Renal Fossa	Neck
	5	0	29	Renal Fossa	Neck
6	4	3	27	Neck	Renal Fossa
7	3	0	22	Neck	Renal Fossa
8	4	0	23	Neck	Renal Fossa
9	6	2	17	Renal Fossa	Neck
10	10	1	41	Renal Fossa	Neck
11	6	1	11	Renal Fossa	Neck
12	14	5	24	Renal Fossa	Neck
13	3	1	21	Renal Fossa	Neck
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*A day of function implies a urinary output by the transplant of 50 cc or more

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arteritis are prominent microscopic features of primary transplantation, whereas extensive hemorrhage and tubular necrosis are the dominant findings in the secondaries Microscopic evidence of infarction was found in 4 instances of secondary homotransplantation but was not usually seen in primary homotransplants removed at the time function ceased It is emphasized that all the 15 primary and secondary homotransplantations in this series were technical operative successes with less than 30 minutes of renal anoxia widely patent vessels after operation, and in all but 2 instances secretion of clear urine within 15 minutes after completion of the operation The secondary homotransplantations in experiments 7 and 8 never put out any urine although renal anoxia times were around 20 minutes and vascular anastomoses were satisfactory The urine from secondary homotransplants was never free from microscopic hematuria although there were often days of blood free urine from primary transplants The characteristic urine odor was often absent from the fluid secreted by the secondary homotransplant

DISCUSSION

It is readily apparent that a secondary homotransplanted kidney secretes for a shorter interval than does the primary and tends to have a different pathological picture [3, 4] However, it is very difficult to be certain of more than these generalizations because of the great variation in the experiments It is our feeling that many of the variations in the course of renal homotransplants *in this study are based on antigenic differences* and similarities in the animals used

Only 1 of the secondary transplants in this series functioned as long as did the primary and resembled the primary both grossly and microscopically There were 4 infarcts in the secondary homotransplant group and in only 2 of these was thrombosis of the renal artery observed In the other 2 cases, the renal artery and vein were widely patent as far as they could be traced into the renal parenchyma The absence of blood flow through the kidneys may be explained by an intense spasm of the smaller intrarenal vascular branches Further evidence for the importance of the vascular element in the secondary homotransplant picture is the presence of massive hemorrhages in most microscopic secondaries sections

In only a few cases were round cells observed in the secondary transplants in contrast to the very large number consistently found in the primary It has been suggested that the mononuclear cell is of transplant origin and does not play a vital role in the homotransplant rejection [7, 8] There is usually progressive proliferation of mononuclear cells within transplanted tissues or organs however

Dempster indicates that a primary feature of the secondary homotransplant reaction is the appearance of a protein precipitate in the subcapsular glomerular space In the 15 secondary homotransplants in this series however this feature was observed infrequently It appears in fact that the glomeruli are only minimally involved in both primary and secondary homotransplant reactions retaining normal configuration clear capsular spaces and normal vascularity in the majority of instances The picture of tubular necrosis and interstitial hemorrhage so frequent in the secondary transplant of this study makes the vascular system distal to the glomeruli a suspected site of antigen antibody reaction Such a reaction resulting in

capillary rupture and tubular anoxia would seem best to explain the pathological findings observed

Varying the site of transplantation did not change the rapidity of destruction of the secondary homotransplants. This is in keeping with Medawar's observation that the local state of immunity is of little significance in destruction of second set homografts.[1] One secondary transplant performed 133 days after the primary transplant only functioned for 2 days compared with 7 days for the primary, showing that at least in this animal the immune state persisted over 4 months. However other workers have shown that there is a progressive decline in immunity and that after a sufficient interval secondary homotransplants will behave like primaries.[9, 10] These studies add additional indirect evidence in support of the immune theory of homograft destruction.

CONCLUSIONS

1 Secondary kidney homotransplants from the same donor to the same host functioned for a much shorter interval than did the primary homotransplant.

2 Primary kidney homotransplants are much enlarged and edematous grossly compared to slightly enlarged and hemorrhagic secondaries. The predominant microscopic features of secondary kidney hemotransplants are hemorrhage and tubular necrosis while round cell proliferation and necrotizing arteritis are the outstanding characteristics of the primary reaction. The glomeruli of both primary and secondary homotransplanted kidneys are the least involved of the renal elements.

3 Varying the site of transplantation or the time interval between transplants up to 133 days does not alter the accelerated secondary homotransplant reaction. These studies offer further support for the immune theory of homotransplant destruction.

REFERENCES

1 Medawar P B The behavior and fate of skin autografts and skin homografts in rabbits J Anat Lond *78* 176 199 1944
2 Dempster W J Kidney homotransplantation Brit J Surg *40* 447 465 1953
3 Dempster W J The relationship between the antigens of skin and kidney of the dog Brit J Plastic Surg *5* 228 237 1953
4 Simonsen M Buemann J Gammeltoft A Jensen F and Jorgenson K Biological incompatibility in kidney transplantation in dogs I Experimental and morphological investigations Acta path microb scand *32* 1 1953
5 Simonsen M and Sorenson F Homoplastic kidney transplantation in dog Acta chir scand *99* 61 72 1949
6 Hume D M and Egdahl R H Progressive destruction of renal homografts isolated from the regional lymphatics of the host Surgery *38* 194 1955
7 Dempster W J Lennox B and Boag J W Prolongation of survival of skin homotransplants in the rabbit by irradiation of the host Brit J Exp Path *31* 670 679 1950
8 Darcy D A A study of the plasma cell and lymphocyte reaction in rabbit tissue homografts Proc Roy Soc Ser B Biol Sc Lond *236* 463 2503 1952
9 Lehrfeld J W Taylor A C and Converse J M Observations on second and third set skin homografts in the rat Plastic & Reconstr Surg *15* 74 76 1955
10 Billingham R E Brent L and Medawar P B Quantitative studies on tissue transplantation immunity Proc Roy Soc Ser Biol Sc Lond *143* 58 80 1954

DISAPPEARANCE OF THYROID HORMONES FROM PLASMA OF DOGS*

TILLMAN M MOORE JR AND LAWRENCE W O NEAL

The endogenously produced circulating thyroid hormone is chiefly l thyroxine bound to plasma proteins [1-3] There is evidence that l thyroxine is changed to tri iodothyronine in tissues[4] and that the latter may be the naturally active hormone at the cellular level Although the effects of the 2 hormones are qualitatively similar, there are marked quantitative differences Thus tri iodothyronine is 3 to 8 times more active than thyroxine in increasing oxygen consumption [5,6] in stimulating amphibian metamorphosis[7] and in interfering with the action of goitrogens [8] Although the effects of tri iodothyronine are more pronounced than those of thyroxine the action of tri iodothyronine is of shorter duration [9,10] These reported differences suggested that tri iodothyronine might be removed from plasma and utilized at a more rapid rate than thyroxine

This report presents a study of the disappearance of protein bound iodine from plasma after intravenous injection of l thyroxine or l tri iodothyronine in thyroidectomized dogs Since the plasma protein bound iodine is proportional to the plasma concentration of these substances a comparison of the relative rates of their removal from plasma can be made

METHOD

Healthy young adult mongrel dogs were anesthetized with intravenous pentobarbital and subjected to total thyroidectomy with preservation of their parathyroid glands These animals were maintained on Purina dog chow which contains 0 5 per cent iodized salt After thyroidectomy plasma protein bound iodine concentrations in these dogs fell to stable low values within an interval of 5 days Single intravenous injections of sodium l thyroxine (Sigma) or sodium l tri iodothyronine (Smith Kline and French) were given after a minimum interval of 1 week following thyroidectomy Serial venous blood samples were then drawn from the opposite leg until the protein bound iodine concentration reached the pre injection (equilibrium) value Protein bound iodine determinations were done by the chloric acid method as modified in this laboratory [11] In our hands the standard deviation of duplicate analyses from their means using this method is ± 0 5 μg per cent Protein bound iodine concentrations are expressed in μg per 100 ml of plasma

RESULTS

Four dogs were given 6 single intravenous injections of 500 μg of l thyroxine Five dogs received l tri iodothyronine 2 of these received 1000 μg and the remainder were given 500 μg Serial blood samples were drawn beginning at 10 minutes and continuing until low concentrations of plasma protein bound iodine at or near equilibrium values were obtained The results are shown in Figure 1 The pre injection mean protein bound iodine concentrations in these animals was 1 2 ± 0 5 μg per cent

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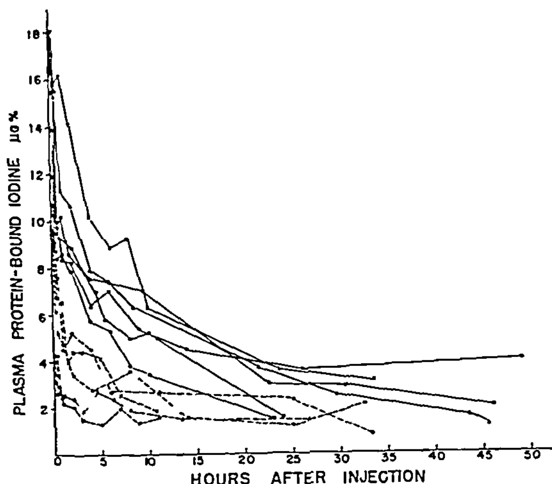


Fig 1 Plasma protein bound iodine after intravenous injection of l thyroxine (solid lines) and of l tri iodothyronine (broken lines) in thyroidectomized dogs

It will be noted that this equilibrium range was reached in the dogs receiving tri iodothyronine between 10 and 15 hours after injection and between 25 and 50 hours in the dogs receiving thyroxine

Comparison between the 2 sets of data was possible only after correction for differences in dosage and in chemical recovery (100 per cent for thyroxine and 70 per cent for tri iodothyronine) and for differences in iodine content of the hormones Na l thyroxine contains 63 6 per cent iodine by weight and Na l tri iodothyronine contains 56 6 per cent The theoretical plasma protein bound iodine concentration at conditions of instantaneous plasma distribution (t_o) was calculated for each injection assuming a uniform blood volume of 90 ml per kg of body weight

$$PBI_o\ (\mu g\ \%) = \frac{\text{Injected dose}\ (\mu g)\quad \text{I fraction in hormone}\quad \text{recovery}\ (\%)}{\text{Plasma volume (ml)}}$$

The calculated PBIt_o to values did not differ significantly from those obtained by extrapolation

The percentages of the PBIt_o concentrations remaining in 100 ml of plasma at varying times after injection are shown in Table I The greatest rate of disappearance of both hormones occurred in the first 10 minutes However tri iodothyronine disappeared from plasma at a more rapid rate

Table 1 The Percentage of PBI_{131} (± Standard Error of Mean) Remaining in 100 ml of Plasma at Varying Times After Injection

	TIME								
	10 MIN	20 MIN	30 MIN	1 HOUR	2 HOURS	4 HOURS	6 HOURS	10 HOURS	20+ HOURS
L Thyroxine	78.1±7.6	61.8±5.7	59.2±7.0	53.8±5.4	47.0±5.0	34.1±3.2	31.8±3.0	25.1±3.6	9.0±2.7
L Triiodothyronine	33.1±10.0	27.1±7.8	20.0±5.7	13.3±2.6	13.1±1.6	9.7±3.4	7.9±0.9	3.7±1.4	1.6±1.3
Level of Confidence of Difference (p)	<0.01	<0.01	0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.02

throughout the experiment For example during the first 10 minutes 66 6 per cent of the injected tri iodothyronine but only 21 6 per cent of the thyroxine disappeared from plasma Of the amounts *present in plasma at 10 minutes* 90 1 per cent of the tri iodothyronine and only 68 2 per cent of the thyroxine had been removed by the tenth hour

DISCUSSION

The results of this experiment indicate that l tri iodothyronine disappears from the plasma of the dog more rapidly than does l thyroxine Both the initial distributional phase and the later phase attributed to metabolic and excretory disposal appear to be more rapid with tri iodothyronine The differences in the rate of distribution might be due to differences in the rate and completeness of protein binding or in the rate of transit across cell membranes Either more rapid metabolic destruction or more rapid excretion could account for the more rapid disappearance of tri iodothyronine after distribution is complete Because of these variables only limited information about peripheral metabolism of these hormones could be obtained in this study However it is of interest that the rates of removal from plasma of these hormones are roughly proportional to their effect in stimulating metabolism The more rapid rate of disposal of l tri iodothyronine is also compatible with its briefer duration of action In separate experiments[1] we have found that d l thyroxine with half the metabolic activity of l thyroxine disappears from plasma more slowly than the latter hormone

The dog disposes of thyroid hormones much more rapidly than does the human This has been observed previously with d l thyroxine[1, 13] and is reported here for l thyroxine and l tri iodothyronine If the data are plotted semi logarithmically biological half times for the rates of disposal from plasma can be estimated These half times in the dog are 10 1 hours for l thyroxine and 4 98 hours for l tri iodothyronine Comparatively these half times in the euthyroid human are 6 7 days and 2 7 days[10]

SUMMARY

(1) Intravenous injections of l thyroxine and l tri iodothyronine were made in thyroidectomized dogs and serial plasma protein bound iodine concentrations determined

(2) L tri iodothyronine disappears from the plasma of the dog more rapidly than l thyroxine

REFERENCES

1 Rosenberg I N The nature of the circulating thyroid hormone in Graves disease J Clin Invest *30* 1 9 1951

2 Gross J Leblond C P Franklin A E and Quastel J H Presence of iodinated amino acids in unhydrolysed thyroid and plasma Science *111* 605 608 1950

3 Tong W Taurog A and Chaikoff I L Nature of plasma iodine following destruction of the rat thyroid with I^{131} J Biol Chem *195* 407 413 1952

4 Kalant H Lee R and Sellers E A Metabolic fate of radioactive thyroid hormones in normal and propylthiouracil treated rats Endocrinology *56* 127 134 1955

5 Blackburn C M McConahay W M Keating F R Jr and Albert A Calorigenic effects of single intravenous doses of l tri iodothyronine and l thyroxine in myxedematous persons J Clin Invest *33* 819 824 1954

6 Lerman J The physiologic activity of l tri iodothyronine J Clin Endocr Metab *13* 1341 1346 1953

7 Bruice T C Winzler R J and Kharasch N The thyroxine like activity of some new thyroxine analogues in amphibia J Biol Chem *210* 1 9 1954

8 Fleming A F and Holtkamp D E Calorigenic and antigoitrogenic actions of l tri iodothyronine and l thyroxine in thyroidectomized and intact rats Proc Soc Exp Biol N Y *83* 875 879 1953

9 Anderson B C Potency and duration of action of tri iodothyronine and thyroxine in rats and mice Endocrinology *54* 659 665 1954

10 Sterling K Lashof J C and Man E B Disappearance from serum of l thyroxine and l tri iodothyronine in euthyroid subjects J Clin Invest *33* 1031 1035 1954

11 O Neal L W and Simms E S Determination of protein bound iodine in plasma or serum Amer J Clin Path *23* 493 505 1953

12 O Neal L W Plasma protein bound iodine after intravenous injection of thyroxine in thyroidectomized dogs Endocrinology *53* 358 366 1953

13 Danowski T S Man E B and Winkler A W Tolerance of normal and of thyroidectomized and of thiourea or thiouracil treated dogs to oral dessicated thyroid and to intravenous thyroxine Endocrinology *38* 230 237 1946

RENAL HOMOTRANSPLANTATION IN IDENTICAL TWINS*

JOSEPH E MURRAY JOHN P MERRILL AND J HARTWELL HARRISON

This is a report of the successful kidney transplantation from one identical twin to another with good renal function persisting after 9 months

Previous attempts at renal homotransplantation both clinically and experimentally had been unsuccessful with the one exception between dizygotic bovine twins in which a kidney transplant has survived and functioned for over a year Success in that instance presumably resulted from the production of an acquired mutual tolerance to each other s tissues by the mingling of fraternal protein in the common placental circulation [1] In all other instances failure of the transplanted kidney occurs by what appears to be an immune or antigen antibody like reaction between donor tissue and recipient The microscopic pattern of the rejected kidney indicates that the homograft itself is actively reacting against the recipient to a greater degree than had been suspected by earlier investigators [3]

Transplantation of kidneys in dogs rarely maintain function for more than 10 to 14 days in spite of vigorous attempts to alter the rejection response [4 5 6] Although human renal homotransplants have functioned for a longer period of time 1 for as long as 5½ months permanent survival has not occurred and the clinical and microscopic pattern of rejection is similar to that in the experimental animal [7]

There are however several experimental observations which made success between identical twins seem likely and justified the removal of a normal kidney from a healthy donor (1) Immunologic and genetic similarity apparently accounts for the permanent survival of *skin* homografts between identical twins [8] (2) When skin or kidney homografts are carried out between antigenically dissimilar individuals the early function and the

*From the Surgical and Medical Services Peter Bent Brigham Hospital and the Laboratory for Surgical Research Harvard Medical School Boston Mass Grant acknowledgments Medical Research and Development Board Office of the Surgeon General Department of the Army U S Public Health Service American Heart Association John A Hartford Foundation Inc

histological picture of rejection of each appear similar[9] (3) Skin and kidney homografts possess a common antigen which can sensitize a recipient to a subsequent homograft of either tissue from the same donor[2,3] This further suggests that skin and kidney grafts might behave similarly (4) We had established to our own satisfaction that renal *autografts* had normal function indefinitely in animals This observation is important because presupposing initial success of the transplant between antigenically similar (identical) twins a second problem to be weighed was the permanency of such function Lacking reported instances of adequate functional studies in long term renal autografts we observed in our own experiments that permanently successful function of a single life sustaining renal autograft resulted from the use of a recipient site which allows direct implantation of the ureter into the bladder which has a normal thermal environment and which permits gravity drainage from the renal pelvis to the bladder

This laboratory technique proved adaptable for use in man provided that the left kidney is placed into the right iliac area or the right kidney into the left iliac fossa thus reversing the normal anteroposterior relationship of the artery vein and ureter

The natural site for the homograft the renal fossa has 2 disadvantages First it requires simultaneous nephrectomy thus increasing the magnitude of the operation Secondly it necessitates an uretero ureteral anastomosis with the possibility of subsequent stricture formation because the length of transplanted ureter vascularized via the renal pedicle is too short to reach the bladder

The upper thigh the site of the 13 previous homotransplants was not used because it requires a skin ureterostomy with the possibility of subsequent ascending infection In addition it creates a problem in the collection of urine

The selected site retroperitoneally within the pelvis utilizing the iliac vessels allows implantation of the short ureteral segment directly into the bladder and places the kidney on its natural thermal environment Furthermore gravity drainage of the renal pelvis and the ureter approaches normal physiological conditions

History and Physical Examination Richard H a 24 year old white single male was apparently well until August 1953 when he noticed some puffiness about the eyes and on a routine physical examination elevation of blood pressure was noted Several months study at another hospital revealed an elevated blood pressure and a persistent 2 to 3+ proteinuria a fixed specific gravity of the urine at 1 010 with red cell casts PSP excretion was less than 1 per cent in 2 hours and an intravenous pyelogram revealed no dye excretion on either side In the following months he became chronically ill The blood pressure was 180/120 with hypertensive retinal changes The blood urea nitrogen was 185 mg per cent He required maintenance on intravenous infusions and became increasingly drowsy disoriented and irritable and had several generalized convulsions Since the patient had a twin brother Ronald it was suggested by Dr David C Miller of the Public Health Service that the possibility of homotransplantation of a kidney should be considered For the investigation of this possibility he was transferred to the Peter Bent Brigham Hospital on October 26 1954

On admission the patient appeared thin pale drowsy and extremely

disoriented and restless. On the fourth hospital day the patient was treated with the artificial kidney for a 4 hours period. Good chemical response was obtained and 36 hours later the patient was rational and cooperative and able to eat. On the fifteenth hospital day full thickness skin grafts 2½ by 2½ cm. were transferred between the twins. A control autograft was placed proximally and the homograft was placed 1 cm. distally, allowing a bridge of normal tissue to intervene between the 2 grafts. The following day the patient was discharged feeling relatively well. On December 13, 1954 he was re-admitted to the Peter Bent Brigham Hospital because of marked increase in the signs and symptoms of his congestive failure. His blood pressure was 220/116, there was 3+ pitting edema of the lower legs up to the knees, bilateral basilar rales, and the liver edge was tender and 1 cm. below the right costal margin. A chest film showed marked cardiac enlargement with evidence of fluid at the right base. On December 16 the skin grafts were biopsied; grossly and microscopically the homograft was indistinguishable from the control autograft. Because of this evidence of tissue compatibility and other ancillary observations that the twins were monozygotic, on December 23 a normal left kidney was removed from Ronald (the healthy twin) and transplanted to Richard. (Previous hospitalization had disclosed the absence of discoverable disease in Ronald and confirmed the presence of 2 normally functioning kidneys free of infection.)

Operation. In adjacent operating rooms a left nephrectomy was started on the donor (by J. H. H.) simultaneously with this operation on the recipient (by J. E. M.). An oblique incision was made in the right lower quadrant just above and parallel to the inguinal ligament. The retroperitoneal area was entered exposing the right iliac vessels. The hypogastric artery was isolated and several of its branches ligated and divided down to the obliterated portion of the hypogastric. The common iliac vein was dissected free and isolated.

The operation was started at 8:15 a.m. The anastomotic site was ready at 9:50 a.m. The donor kidney was brought in at 9:53 a.m. The end-to-end anastomosis between the hypogastric artery and the renal artery was completed at 10:40 a.m. The end-to-side anastomosis between the renal vein and common iliac vein was completed at 11:15 a.m. Total ischemia of the kidney was 1 hour and 22 minutes. The entire kidney became turgid and pink immediately on release of the arterial clamp. Therefore a small artery which appeared to be an accessory renal artery was ligated rather than anastomosed.

At this point, J. H. H. who had completed the nephrectomy on Ronald assisted in the implantation of the ureter. The ureter was led into the bladder through a submucosal tunnel and a mucosa-to-mucosa suture carried out. At this time clear urine was flowing rather copiously from the ureteral catheter.

The wound was then inspected. The kidney lay rather neatly in its new site except that it projected forward where the lower pole impinged upon the iliac crest. The upper pole of the kidney was fixed by sutures to prevent its rotation. The kidney was well vascularized everywhere with free bleeding from the surface and hilum as well as from the ureter. There was a brisk flow of urine noted from the ureteral catheter.

A 1 layer closure of the internal oblique muscle, the external oblique

fascia, the subcutaneous tissue and the skin was then carried out Heparin was not used The donor kidney was not irrigated or perfused with antibiotics or any other solution Total operating time 5 hours 30 minutes

Postoperative Course The postoperative course of the donor was uneventful and he was discharged on the 11th hospital day The recipient tolerated the operative procedure well On the 13th postoperative day the intravenous injection of indigo carmine showed prompt appearance in good concentration from the transplant The palpable mass of the homograft in the right lower quadrant began to hypertrophy and ultimately enlarged to one third again its normal size On discharge from the hospital on the 37th postoperative day the patient had gained 11 lb and was edema free and the blood urea nitrogen had gradually fallen to 11 mg per cent The resting blood pressure was 120/60, chest clear, and heart size normal The phenolsulfonphthalein excretion was 18 per cent in 15 minutes and 18 per cent in 2 hours

The patient s left kidney was removed 3 months post transplantation and the right kidney 5 months post transplantation (by J H H) On gross appearance they were small scarred, with markedly diminished renal cortical tissue Microscopically the appearance was a diffuse advanced chronic glomerulonephritis

At present the patient is asymptomatic and carrying on unlimited activity with no apparent disability His appetite is good and he has gained 25 lbs since the original operation The blood pressure ranged from 125/70 to 146/82 and his blood chemistries are normal

DISCUSSION

The removal of the 2 damaged kidneys was accomplished for 4 reasons The evidence from intravenous pyelography that the renal homograft was functioning well the possibility of infection of the graft from the 2 remaining diseased kidneys the experimental evidence suggesting that a non functioning or infected kidney may ultimately interfere with the normal function of the normal kidney particularly with regard to its role in renal hypertension and the data indicating almost total lack of function in the 2 diseased organs

The survival of the renal homograft for this period of time with continuing good function indicates the complete lack of a rejection response by the host and demonstrates that renal transplantation is a technically feasible procedure The implications of the dramatic response of the malignant hypertensive disease to the transplantation of a normal kidney should carry considerable weight in future thinking about the renal mechanism in human hypertension Why one identical twin and not the other should develop glomerulonephritis and whether the kidney of the unaffected twin transplanted into the diseased recipient will be susceptible to further attacks are questions still to be answered

REFERENCES

1 Simonsen M Acquired immunity concept in kidney homotransplants Ann N York Acad Sc *59* 448 1955
2 Simonsen M Buemann J Gammeltoft A Jensen F Jorgensen K Biological incompatibility in kidney transplantation in dogs Acta path microb scand *32* 1 1953
3 Dempster W J Kidney homotransplantation Brit J Surg *40* 447 1953

4 Persky L Jacob S Effect of ACTH and cortisone on homogenous kidney transplants Proc Soc Exp Biol N Y 77 66 1951
5 Dempster W J The effects of cortisone on the homotransplanted kidney Arch Internat pharm dyn Gand 95 253 1953
6 Murray J E Lang S Miller B F Observations on the natural history of renal homotransplants in dogs in Surgical Forum 1954 Philadelphia W B Saunders Co 1954 p 241
7 Hume D M Merrill J I Miller B F Thorn G W Experiences with renal homotransplantation in the human Report on 9 cases J Clin Invest 34 327 1955
8 Brown J B Homografting of skin with report of success in identical twins Surgery 1 559 1937
9 Dempster W J Problems involved in the homotransplantation of tissues with particular reference to skin Brit M J 2 1041 1951

A SINGLE INJECTION METHOD OF MEASURING HUMAN RENAL PLASMA FLOW*

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LEO A SAPIRSTEIN AND ROBERT N WATMAN

The estimation of renal plasma flow as bladder clearance of sodium para amino-hippurate (PAH) suffers the disadvantage that in oliguric and anuric patients the urinary clearance does not represent the actual renal elimination of the test substance In order to measure the renal plasma flow in conditions of anuria and oliguria it is necessary to estimate the renal elimination of PAH from the circulating blood volume This measurement may be made either by determination of the infusion rate when plasma PAH concentration equilibrium has been attained or by analysis of the disappearance curve after a single intravenous injection of PAH [1-3]

In the present study of 10 normal subjects the infusion clearance of PAH has been compared with the clearance estimated from such a disappearance curve The results indicate that the single injection technique yields values in fair agreement with the infusion method and that it may have applicability in the clinical determination of renal plasma flow

METHOD

The subjects were patients on the surgical service at the Ohio State University Medical Center who had no clinical evidence of renal disease The patients received a single rapid intravenous injection of 3 mg of PAH per kg of weight No precautions were taken to prehydrate the patients or to control diet After injection venous blood samples were taken at 5 *minute intervals for 50 minutes In several experiments the first 3* samples were taken at 3 minute intervals and thereafter at 5 minute intervals for 50 minutes A blood sample was taken from each subject prior to the injection and served as a blank determination on PAH In each experiment an aliquot of the solution injected served as the standard when properly diluted

*From the Departments of Surgery and Physiology The Ohio State University Columbus Ohio This study was supported in part by grants in aid from the Graduate School of the Ohio State University and the Central Ohio Heart Association

PAH analysis was conducted by the method of Smith *et al*,[4] all colors being read in a Beckman D U Spectrophotometer at a wave length of 5350 angstroms and using 5 cm cuvettes * Immediately after the last sample, a PAH primer of 30 mg was given and an infusion of 1 5 mg per minute of PAH was started through a constant infusion pump Blood samples were taken at 10, 50 and 60 minutes after the onset Equilibrium had been attained in all subjects of the reported series within 50 minutes The clearance of PAH was calculated in the single injection technique by the use of formula 1 (Figure 1) and the curve constants obtained from a double

Fig 1 Illustrates the equations used in calculating the clearance volumes of distribution and intercompartmental clearance of PAH following a single intravenous injection P is the effective renal plasma flow

1. $P = \frac{I \gamma_1 \gamma_2}{A \gamma_2 + B \gamma_1}$

2. $V_1 = \frac{I}{A+B}$

3. $\text{alpha} = \frac{V_1}{A+B} [A \gamma_1 + B \gamma_2] - P$

4. $V_2 = \frac{\text{alpha } P}{V_1 \gamma_2 \gamma_2}$

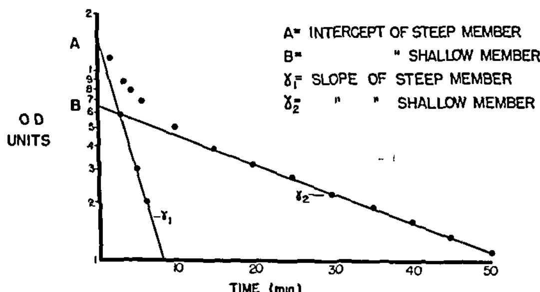


Fig 2 Disappearance curve for PAH *after single* intravenous injection in man Where A is the intercept on the concentration ordinate of the steep member of the pair of lines constructed that of the shallow member B Gamma and Gamma, are the slopes of the steep and shallow membered lines respectively

* It is necessary to use 5 cm cuvettes for the single injection clearance determination because of the extremely low *concentrations of PAH found* at the end of the clearance period This cannot be remedied by the use of larger doses for such large doses result in exceeding the tubular maximum for PAH *in the* early portions of the curve and vitiate the results entirely The doses recommended allow a margin of safety of 2 if the PAH is distributed only in the plasma and a much larger margin of safety if it penetrates the extra vascular fluid

Table 1 Clearance of PAH following a Single Intravenous injection and After Constant Infusion in 10 Clinical Patients

NO	NAME	WEIGHT KG	ALPHA CC/MIN	V_1 LITERS	V_2 LITERS	PCI CC/KG/MIN	Ist CC/KG/MIN
1	M Z	30	392	4 17	3 68	6 8	9 4
2	M C	59	510	10 3	7 4	13 6	16 6
3	E F	70	—	——	0	9 7	9.5
4	H R	52	595	11 9	9 15	9 6	13 7
5	W S	60	366	9 64	7.58	11 1	9 1
6	W K	34	—	——	0	6 9	6 3
7	I S	69	538	3 99	5 26	7 6	6 8
8	G T	92	—	——	0	5 3	6.2
9	D M	70	65	13.2	4 15	12 6	15 0
10	F F	77	522	6 8	4 62	7 0	6 6
						9 32 Avge	9 92 Avge

In patients E F, W K and G T the disappearance curve for PAH was a single exponential function of time This signifies that the penetration of V_2 was very rapid in relation to the excretory clearance of PAH The value for V_1 in these patients actually represents the sum of V_1 and V_2 The value for alpha is of course indeterminate since no separate V_2 exists

exponential fitting of the plasma concentration time disappearance curve plotted on semi logarithmic paper This was accomplished by making a best visual linear fit to the latter portion of the curve and subtracting the values of this linear fit from the observed data of the earlier portion of the curve If the subtracted values failed to generate a straight line the best fit line was rotated and translated until linearity of the diminished values was obtained A more detailed interpretation of the time decay curve and a derivation of the formulae used in the calculations is available in a previous communication Figure 2 shows a typical double exponential disappearance curve and identifies the symbols used for the curve constants

The infusion clearance value was obtained by dividing the infusion rate in mg per minute by the equilibrium concentration of PAH in mg per ml[1]

RESULTS

The results in 10 patients are presented in Table 1 The curve constants are given and the calculated values of V_1 V alpha and P are shown It must be stressed that V_1 V and alpha are of doubtful significance Their meaning is discussed elsewhere[2,3] The comparison between P as obtained from the single injection and constant infusion methods is presented in the last 2 columns of the table

There is fair agreement between the 2 methods the renal plasma flow by single injection averaging 6 per cent higher than that by constant infusion

DISCUSSION

The clearances which are measured by the 2 methods used are plasma clearance rather than blood clearance and deviate from the latter by the extent to which PAH is acetylated Since it is improbable that there is any other route for the disappearance of PAH from its volume of distribution these clearances could have been equated with the blood clearance by

making a determination of the total rather than conjugated PAH. This was not done in the present experiments since the acetylation of PAH is very small in relation to its renal excretion in normal subjects but such a determination may be necessary when there is profound depression of the renal plasma flow.

The individual discrepancies observed suggest that the plasma clearance of PAH is inherently subject to a greater inaccuracy than the more usually employed bladder clearance. Despite this the determination of plasma clearance offers a clear advantage over the bladder clearance method under clinical conditions in which for any reason there is diminished urine flow. Although it is true that errors may result from the application of these techniques this error does not appear to be sufficiently great to prejudice the value of this procedure as a clinical measurement.

The plasma clearance methods should not be employed in circumstances where the renal plasma flow may be changing in the course of the measurement. Neither is it recommended that it be used without due caution when there are local accumulations of fluid. This method is not applicable to patients receiving sulfa drugs or procaine, as is the case with bladder clearance measurements.

SUMMARY

A method has been described for the estimation of effective renal plasma flow in human subjects by analysis of the disappearance curve of PAH following a single intravenous injection. The clearance of PAH by this method in 10 clinical subjects averaged 6 per cent higher than the PAH clearance by the constant infusion method which was used as a reference standard.

The applicability of this method to the estimation of renal plasma flow in oliguria and anuria is discussed.

REFERENCES

1. Berger E Y, Farber S J and Earle D P. Comparison of constant infusion and urine collection techniques for measurement of renal function. J Clin Invest 27 710 716 1948
2. Sapirstein L A, Vidt D G, Mandel M J and Hanusek G. The volumes of distribution and clearances of intravenously injected creatinine in the dog. Am J Physiol *181* 330 1955
3. Mandel M J, Vidt D J and Sapirstein L A. Disappearance of PAH from the plasma of the dog after a single intravenous injection. Am J of Physiol (In Press)
4. Smith W W, Finkelstein N and Smith H W. Renal excretion of hexitols and their derivatives and of endogenous creatinine like chromogen in dog and man. J Biol Chem *135* 231 1940

THE EXPERIMENTAL TRANSPLANTATION OF REGENERATED AND FETAL THYROID TISSUE TO THE SPLEEN*

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Regeneration begins in the thyroid tissues of the cat soon after subtotal thyroidectomy Microscopic examination of this regenerating thyroid tissue reveals a marked departure from the orderly cytology of the normal gland [1]

These experiments were designed to demonstrate alterations in thyroid gland function and histology following transplantation of regenerated or fetal thyroid tissues to the spleen

METHOD

Fifteen young healthy adult cats were selected for the experiments Eight of the animals were male and 7 were female

Subtotal thyroidectomy was performed under intravenous sodium pentobarbital anesthesia in 13 of these animals An estimate was made of the weight of remaining thyroid tissue in each animal This was done by weighing a segment from the excised thyroid lobe which approximated the size of the thyroid remnant in the neck (Fig 1a) In the group of 13 cats the estimate of residual thyroid tissue ranged from 32 to 65 mg The weight of the average thyroid remnant was 52 mg Three weeks later the thyroid area of these cats was again explored under sodium pentobarbital anesthesia All remaining thyroid tissue was excised and immediately weighed under aseptic conditions The weight of these specimens of regenerating

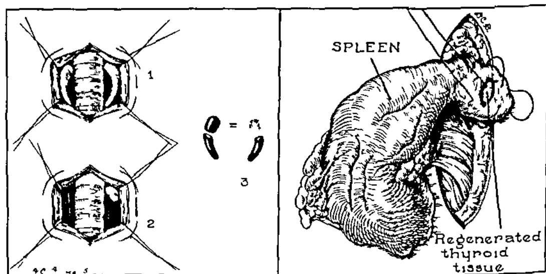


Fig 1 a Diagram illustrating (1) the normal cat thyroid gland in situ (2) the amount of residual thyroid tissue left after subtotal thyroidectomy and (3) the method for estimating residual thyroid tissue after subtotal thyroidectomy (This was done by weighing a portion of the excised lobe that was similar in size and shape to the residual tissue) b Diagram indicating the technique of transplantation of regenerating thyroid tissue to the spleen

*Supported by the Christine Breon Fund for Medical Research From the Surgical Research Laboratories of the University of California School of Medicine San Francisco

thyroid tissue ranged from 67 to 116 mg with an average weight of 101 mg for the entire group A weighed amount of each regenerating thyroid specimen was then inserted into the spleen of the cat from which it had been excised (Fig 1b)

In 2 other cats total thyroidectomy was performed under sodium pento-barbital anesthesia as a one stage procedure At the same operation fetal cat thyroid tissue that had been obtained the same day from unborn kittens that were approximately 2 weeks from birth was placed in the spleens of these animals

During the next 18 months records of body weight appetite appearance pregnancy in females and occurrences of illness were kept for each cat Complete autopsy was performed on all animals expiring during this period At the end of the period the surviving animals and 3 normal controls were each given 100 mc. of I^{131} intramuscularly The uptake of the radioactive isotope was measured in each animal 12 hours 30 hours and 170 hours later The sites of measurement were the anterior cervical region left abdominal upper quadrant and right thigh Background counts of radioactivity and standardization of the I^{131} sample were made at frequent intervals and corrections made for their effects upon the study The cats were then sacrificed and complete autopsy was performed All spleens were examined microscopically for the presence of thyroid tissue

RESULTS

Seven cats were alive at the end of the 18 month period of observation Three were females and 4 were males All were in good health Of the 8 cats that had died one had expired on the operating table during subtotal thyroidectomy and the others had died during an epidemic of distemper The spleens of the distemper victims contained viable thyroid tissue in 6 instances However none of these animals had survived longer than 4 weeks after the transplantation procedure and they were not included in the final results of the study

The 7 surviving cats included the 2 animals that had received fetal thyroid transplants to their spleens Examination of the clinical records of all 7 failed to reveal evidence of deficient thyroid function All had maintained body weight and appetite except during the distemper epidemic which had attacked all the animals in the colony The condition of the coat of each animal was good and muscle tone and mass had remained excellent The 3 females had undergone a total of 5 pregnancies with delivery of apparently normal viable kittens in each instance

The uptake of I^{131} by these experimental animals was maximal over the left upper quadrant of the abdomen in 6 of the 7 cats This observation was true for the three measurements carried out in each animal The average uptake in these 6 cats 30 hours after injection of I^{131} was 31 per cent by the left upper quadrant of the abdomen 10 per cent by the anterior cervical region and 5 per cent by the right thigh The one animal that was the exception to these observations had received a fetal thyroid transplant to the spleen This cat was found to have residual cervical thyroid tissue at autopsy The 3 control animals showed greatest uptake of I^{131} by the thyroid region For them the average uptake was 15 per cent by the anterior

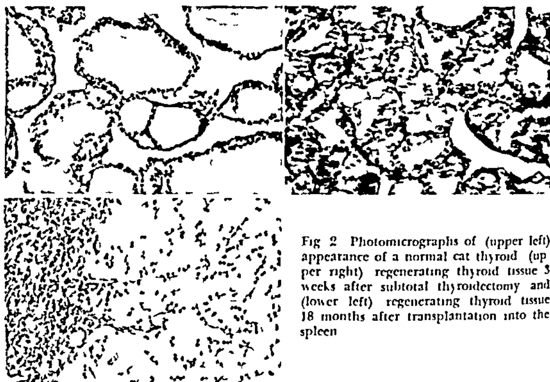


Fig 2 Photomicrographs of (upper left) appearance of a normal cat thyroid (upper right) regenerating thyroid tissue 3 weeks after subtotal thyroidectomy and (lower left) regenerating thyroid tissue 18 months after transplantation into the spleen

cervical region 17 per cent by the left upper quadrant of the abdomen and 3 3 per cent by the right thigh

Autopsy revealed viable thyroid tissue within the spleens of 6 of the experimental animals Residual thyroid tissue could not be identified within the necks of these animals The seventh cat as previously indicated did have residual cervical thyroid tissue This animal had received a fetal transplant which could not be identified within the spleen at autopsy

Microscopic sections were prepared of the spleens of the 6 animals that had shown maximum I^{131} uptake over the left upper quadrant of the abdomen The thyroid tissue within these spleens was marked by the formation of small, irregular follicles deficient in colloid The cells of these follicles were columnar Their nuclei were centrally placed and were irregular in size and deeply basophilic Numerous mitoses were observed within these nuclei Cytoplasm was abundant and granular The overall appearance was one of marked cellularity Figure 2 compares the microscopic appearance of a normal cat thyroid (Fig 2a) regenerating thyroid tissue 3 weeks after subtotal thyroidectomy (Fig 2b) and the same regenerating tissue 18 months after transplantation to the spleen (Fig 2c)

SUMMARY

1 Successful autotransplantation of regenerating thyroid tissue to the spleen was performed in 5 adult cats

2 Homotransplantation of fetal cat thyroid tissue to the spleen was successful in one adult animal

3 The uptake of I^{131} by all of these animals was maximal over the left upper quadrant of the abdomen when studied 18 months after the transplantation procedure

4 The microscopic appearance of these thyroid tissue transplants is described and illustrated

REFERENCE

1 Johansen R Gardner R F Calante M., Marchi F F Ledwich I W Soley M H Scott K G Miller L F and McCorkle H J An experimental study of thyroid regeneration following subtotal thyroidectomy Surg Gyn Obst 93 303 309 1951

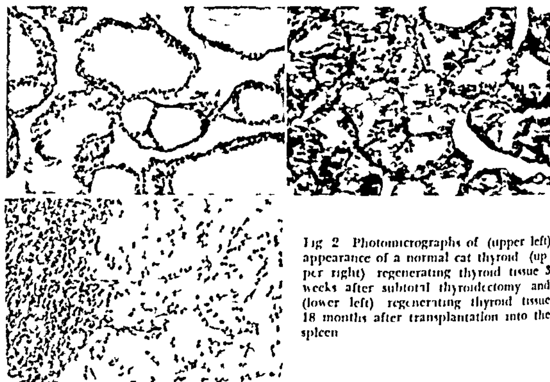


Fig. 2. Photomicrographs of (upper left) appearance of a normal cat thyroid, (upper right) regenerating thyroid tissue 3 weeks after subtotal thyroidectomy and (lower left) regenerating thyroid tissue 18 months after transplantation into the spleen.

cervical region, 17 per cent by the left upper quadrant of the abdomen and 3.3 per cent by the right thigh.

Autopsy revealed visible thyroid tissue within the spleens of 6 of the experimental animals. Residual thyroid tissue could not be identified within the necks of these animals. The seventh cat, as previously indicated, did have residual cervical thyroid tissue. This animal had received a fetal transplant which could not be identified within the spleen at autopsy.

Microscopic sections were prepared of the spleens of the 6 animals that had shown maximum I^{131} uptake over the left upper quadrant of the abdomen. The thyroid tissue within these spleens was marked by the formation of small irregular follicles deficient in colloid. The cells of these follicles were columnar. Their nuclei were centrally placed and were irregular in size and deeply basophilic. Numerous mitoses were observed within these nuclei. Cytoplasm was abundant and granular. The overall appearance was one of marked cellularity. Figure 2 compares the microscopic appearance of a normal cat thyroid (Fig. 2a), regenerating thyroid tissue 3 weeks after subtotal thyroidectomy (Fig. 2b) and the same regenerating tissue 18 months after transplantation to the spleen (Fig. 2c).

SUMMARY

1. Successful autotransplantation of regenerating thyroid tissue to the spleen was performed in 5 adult cats.

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4. The microscopic appearance of these thyroid tissue transplants is described and illustrated.

Table 1 Effects of Goitrogens on Pregnancy and Fetus

CASE NO	AGE	OBSTETRIC HISTORY	THYROID SYMPTOMATOLOGY	ANTITHYROID MEDICATION			RESULTS	
				DRUG	DOSAGE	DURATION	MOTHER	FETUS
1 a	29	2 early abortions	*Onset in pregnancy* tremor sweating enlarged gland Exoph thalmous BMR +28	Propyl thiouracil	150 mg	3 months	BMR +10 Uneventful delivery	2700 gm female 36 wks gestation Normal otherwise
1 b	31	See above	14 months remission not medicated Relapse BMR +74 Conceived after stabilized on goitrogen	Propyl thiouracil	250 mg	9 months	Spotting 2nd month Edema last 3 wks Received radioactive iodine 5 mos postpartum	2700 gm male 36 wks gestation Normal otherwise
2	29	1 early abortion 1 normal	Severely toxic enlarged gland BMR +65 Conceived while on goitrogen	Propyl thiouracil	200 mg	9 months	BMR +16 Gradual remission	3270 gm male Normal growth and development
3	29	None	Severely toxic exoph thalmous BMR +64 Hypertension	Propyl thiouracil	150 200 mg	7 months	Decrease toxic symptoms rising B P occasional brief remissions Thyroid ectomy recommended	3000 gm female normal
4	29	None	BMR +35 P R 140 slightly enlarged gland	Propylthioura cil (plus Lugol s temporarily)	200 mg	2½ months	Remission following pregnancy	2900 gm female normal development
5	20	None	Extreme toxicosis congestive failure at 7th month	Methimazole	10 mg	8 days	BMR to +28 Prolapsed cord breech extraction Thyroidectomy 2 mos postpartum	1530 gm prema ture female normal growth & development for 8 months
6	39	1 abortion 1 normal	Enlarged gland tachy cardia nervousness BMR +28	Propyl thiouracil	100 200 mg	8 months	BMR same Symptoms improved Normal deliv	3500 gm female normal

Gynecology and Obstetrics

THE EFFECTS OF GOITROGENIC DRUGS ON PREGNANCY AND THE FETUS*

E RAE HUDSPETH AND R R MARGULIS

The purpose of this study is to determine the effects of the goitrogenic drugs now employed in the treatment of thyrotoxicosis upon pregnancy and the developing fetus

Goitrogens are cyclic compounds related to thiourea (Fig 1) Their action prevents the formation of thyroxin and tri iodotyrosine This is apparently achieved through enzymatic blockage of iodination of tyrosine at the thyroid cellular level The decrease in the circulating thyroid hormone is followed by an increased output in the thyroid stimulating hormone of the anterior pituitary This results in a pronounced hyperplasia of thyroid cells with an increased vascularity of the entire gland [9]

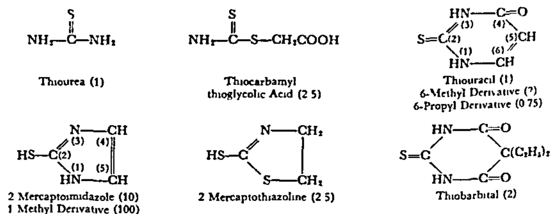


Fig 1 Structure of Antithyroid Drugs Parenthetical figures indicate antithyroid activity in man relative to that of thiouracil From Goodman and Gilman 1955 Table 61 p 1547

METHOD

Seven pregnancies occurring in 6 mothers were studied Each mother was given either propylthiouracil or methimazole (Tapazole[R]) in adequate dosage to control symptoms and signs of thyrotoxicosis The dosages ranged from 100 to 250 mg propylthiouracil and 40 mg methimazole Severity of the conditions varied from mildly elevated basal metabolic rates (+28) fast pulse and minimal toxic symptoms to severe thyrotoxicosis with a basal metabolic rate of +65 and 1 was complicated by congestive heart failure Each patient was followed closely by a team consisting of an obstetrician

*From the Departments of Obstetrics and Gynecology Woman s Hospital Detroit Mich
The authors express appreciation for permission to use case materials of Drs A Seski N Brooks and E C Rupp

That the goitrogens influence the developing fetus has been demonstrated in laboratory animals Thiouracil passes the placental barrier and also is secreted in milk[8] Effects similar to those seen in the adult occur in the fetus The common complication is transient neonatal goiter[11, 14] These goiters are usually asymptomatic However rarely mechanical respiratory difficulties may occur[14] Thus far, one cretin has been reported[11] Slow pulse rate low voltage electrocardiographs and elevation of serum cholesterol have been found[7, 10, 1] Examination of the goiter of a newborn infant whose mother received methylthiouracil throughout pregnancy has shown hyperplasia like that found in adult thyroid tissue after treatment with a goitrogen[15]

Surgery for hyperthyroidism during pregnancy is sometimes detrimental There is increased difficulty of diagnosis of mild thyrotoxicosis during pregnancy and there is also the possibility of a spontaneous remission after termination The usual complications of surgery are to be considered plus the possibility of their being heightened by the increased vascularity and hypervolemia of pregnancy Hypoxia during anesthesia may be more hazardous for the developing fetus than for the adult organism Postoperative abortion has occurred although the rate is apparently no higher than in non operated patients[1]

It is not to be implied that goitrogens are the definitive treatment of choice for thyrotoxicosis However it is felt that the safest method of management during pregnancy would be the use of a goitrogen in conjunction with careful clinical and laboratory controls using frequent basal metabolic rates and being alert for the appearance of the rare severe complication of agranulocytosis Frequently mild thyrotoxicosis which was precipitated by pregnancy may disappear spontaneously after delivery as is seen in case 4 Thyroidectomy should be considered approximately two months postpartum if the signs and symptoms persist

The possibility of concomitant use of iodides during the last two weeks of pregnancy should be considered on the basis that it causes involution of the fetal thyroid obviating a congenital goiter

CONCLUSIONS

Seven pregnancies of 6 mothers receiving goitrogens for thyrotoxicosis have been presented These mothers received from 150 to 250 mg prophylthiouracil or 40 mg methimazole for periods ranging from a few days to the entire period of gestation without ill effects to the mother or the fetus For this reason it is our impression that goitrogens have a definite place in the management of thyrotoxicosis during pregnancy This condition should be handled with team work between the medical endocrinologist and obstetrician Surgical or radiological treatment should be considered two months following delivery if thyrotoxicosis persists The newborn infant should be watched carefully for enlarged thyroid gland and its possible contribution to asphyxia If the mother is continuing the antithyroid medication the infant should not be breast fed

REFERENCES

1 Bell G O Hyperthyroidism pregnancy and thiouracil drugs J Am M Ass *144* 1243 1246

2 Cantcrow A and Trumper M Clinical biochemistry Philadelphia W B Saunders Co 1949 4th Ed p 500

and an internist. Frequent checks were made of white blood counts and basal metabolic rates. Protein bound iodine was not determined since there is a physiologic increase in blood iodine during pregnancy.[2] Radioactive iodine uptake studies were omitted due to the possibility of damage to the fetal thyroid.[4]

RESULTS

The results are shown in Table 1. Age of patients varied from 20 to 39 years. Obstetrical histories showed 4 abortions out of 8 previous pregnancies. Three of the 6 patients were primigravidas. Thyrotoxicosis began during pregnancy in cases 1a and 4. Remission of hyperthyroidism occurred 3 times following termination of pregnancy (cases 1a, 2, 4) with 1 relapsing 14 months later. The symptoms were severe in 4 patients (cases 1b, 2, 3, 5). In each case the mother's pulse rate and metabolic rate returned to normal. Symptoms such as tremor, nervousness and palpitation improved or disappeared. In case 5, digitalization was added to the goitrogen because of cardiac failure. In case 3 additional treatment of hypertension was necessary.

The antithyroid drug propylthiouracil was used with 5 patients. It was given in dosages of 150 to 250 mg. during periods ranging from 2½ to 9 months. Two patients received the drug during the entire period of gestation. Methimazole was given to 1 patient after her first prenatal visit as an emergency medication for severe toxicosis complicated by cardiac failure. She delivered prematurely 8 days later. In none of our cases did the goitrogens used cause allergic symptoms or the severe complication of agranulocytosis.

The infants ranged in weight from 1530 to 3800 gm. Three of the infants were premature but otherwise were healthy. None was born with goiter or other deleterious effect attributable to the goitrogen.

DISCUSSION

As hyperthyroidism occurs only in approximately 0.2 per cent of pregnancies,[5, 10] it is a relatively rare complication. Pregnancy in hyperthyroid patients terminates in fetal losses as high as 50 per cent.[1] These two conditions—hyperthyroidism and pregnancy—have certain physiologic changes in common.

In each hypervolemia develops with an increase in both blood and plasma volume. During the period beginning at about the thirtieth week of gestation, when physiologically in pregnancy the increment of cardiac output and plasma volume is greatest,[3] the additional similar changes due to thyrotoxicosis may occasionally tip the balance of cardiac reserve and result in congestive failure.

Basal metabolic rates remain normal in early pregnancy. However during the latter one half the rate may rise to +20 or +25.[1] This fact must be remembered during the treatment of hyperthyroidism in this stage of pregnancy. Overzealous treatment with the intent to reduce the basal metabolic rate to normal nonpregnant values will result frequently in a hypothyroid state which is accompanied by increased fetal wastage.[1]

From our limited experience and that of others,[9, 13, 16] goitrogens apparently did not affect unfavorably ovulation, conception, gestation, delivery, or postpartum convalescence. In our series cases 1b and 2 conceived while on goitrogens.

slowly at a rate of 30 drops per minute The intravenous drip was preceded by a test dose of ½ minim of pitocin given intramuscularly

Patients in this study were divided into 2 groups

Group A was composed of 20 women at term with uncomplicated obstetrical course all of them were delivered vaginally Labor and delivery in all instances were uncomplicated The average duration of labor was 9 hours The age of patients ranged from 16 to 10 years the average being 24 8 years Nine patients were primiparous and 11 were multiparous

Group B was comprised of 10 patients in whom pitocin induction was chosen in order to bring on regular uterine contractions and/or improve labor The age in this group ranged from 21 to 31 years the average being 29 8 years The duration of labor varied from 15 minutes to 6 hours with an average of 3 hours There were 10 multiparous and no primiparous patients Meperidine hydrochloride and scopolamine hydrobromide were given in moderate doses and were employed in all but 1 patients in Group B Spinal anesthesia with 5 mg of pontocaine or 50 mg of novocaine were used in all but 1 cases

RESULTS

One hundred and thirty five fibrinogen determinations were obtained

Group A In this control group fibrinogen determinations were made in 15 patients according to the Wu Ling method and in 5 according to the Gornall modification The first group will be referred to as Group I and the second as Group II The admission specimen in Group I ranged from 230 mg per cent to 410 mg per cent with an average of 303 mg per cent In Group II the values were from 350 mg per cent to 500 mg per cent the average being 430 mg per cent Thus averages for both determinations were obtained and from now on deviations in further specimens taken at various intervals will be reported besides the actual values in mg per cent as per cent of deviation from the average initial fibrinogen level

Specimen B was obtained at the time of delivery in Group II according to the Gornall modification with a value range from 140 mg per cent to 570 mg per cent The average was 510 mg per cent showing an increase of 18 6 per cent over the initial range

Specimen C was obtained a half hour after delivery in the 20 patients In Group I (15) the levels ranged from 215 mg per cent to 500 mg per cent with an average of 322 mg per cent or 5 9 per cent over the initial value In Group II (5) the fibrinogen varied from 210 mg per cent to 710 mg per cent with an average of 451 mg per cent showing an increase of 4 8 per cent over the initial average

Specimen D was obtained 4 hours after delivery in the 20 patients The first group (15) had a range of 201 mg per cent to 410 mg per cent with an average of 314 mg per cent or 3 6 per cent over the initial value In Group II (5) the range was from 350 mg per cent to 650 mg per cent the average being 467 mg per cent showing an increase of 8 6 per cent of the initial range

Fifteen further determinations were made as Specimen E at 24 hours postpartum and as Specimen F at 4 to 5 days postpartum Determinations were made according to the Wu Ling method In Specimen E the range was from 191 mg per cent to 450 mg per cent the average being 316 mg

3 Caton W L Roby C C Reid D E and Gibson J G Plasma volume and extra cellular fluid volume during pregnancy and the puerperium Am J Obst *57* 471-481 1949
4 Chapman E M Corner G W Jr Robinson D Evans R D The collection of radioactive iodine by the fetal thyroid J Clin Endocr Metab *8* 717 720 1948
5 Dailey M E and Benson R C Hyperthyroidism in pregnancy Surg Gyn Obs *94* 103 109 1952
6 Eaton J C The treatment of thyrotoxicosis with thiouracil Lancet Lond *248* 171 174 1945
7 Elphinstone N Thiouracil in pregnancy Its effect on the fetus Lancet Lond *1* 1281 1283 1953
8 Freiesleben F and Kjerulf Jensen K The effect of thiouracil derivatives on fetuses and infants J Clin Endocr Metab *7* 47 51 1947
9 Goodman L S and Gilman A The pharmacologic basis of therapeutics New York The MacMillan Co 1955 2nd Ed pp 1543 1553
10 Hepner W R Jr Thiourea derivatives and the fetus Am J Obst *63* 869 874 1952
11 Keynes G Obstetrics and gynecology in relation to thyrotoxicosis and myasthenia gravis J Obst Gyn Brit Empire *59* 173 182 1952
12 Morris D Transient hypothyroidism in a newborn infant Lancet Lond *1* 1284 1285 1953
13 Palmer M V Hyperthyroidism and thiouracil Ann Int M *22* 335 364 1945
14 Piper J and Rosen J Management of hyperthyroidism during pregnancy Acta Med Scand *150* 215 222 1954
15 Salm R Thiouracil goiter in a newborn J Obst Gyn Brit Empire *61* 831 832 1955
16 Williams R H Asper S P Jr Rogers W F Jr Myers J D and Lloyd C W Persistence of remissions of thyrotoxicosis after cessation of thiouracil therapy N England J M *236* 737 741 1947

EFFECTS OF INTRAVENOUS PITOCIN ON THE DEVELOPMENT OF HYPOFIBRINOGENEMIA*

ANDRE B MARQUIS AND R R MARGULIS

The purpose of this investigation was to determine the safety of intravenous administration of pitocin from a standpoint of inducing hypofibrinogenemia It has been previously reported that tumultuous labor will introduce thromboplastin into the circulation thus causing intravascular clotting and bleeding

Fibrinogen determinations were therefore made at the time of delivery and at intervals of half an hour and 4 hours after delivery occasionally determinations were also carried out after 24 hours and 4 to 5 days post partum

Initially the Wu Ling method was employed for fibrinogen determinations This was done as part of the control with 15 patients all with uncomplicated spontaneous deliveries The same fibrinogen determinations were made on an additional 5 controls and in 10 women who received intravenous pitocin In the last 2 groups the Gornall method was employed since it was believed to be more sensitive

Posterior pituitary extract (pitocin) was given in the following solution *5 minims of pitocin diluted in 500 cc of 5 per cent glucose in water given*

*From the Departments of Obstetrics and Gynecology Woman s Hospital Detroit Michigan

labor than the control group the average duration of labor was 3 hours as compared with 9 hours in the first group

The possible explanation for the decrease in fibrinogen levels in patients with tumultuous labor and those who received intravenous administration of pitocin is an increase in circulating fibrinolysin or a fibrinogen depletion due to a moderate intravascular clotting

It has been reported that the fibrinolysin level is increased after excessive muscular exercise extreme apprehension and epinephrine administration Intravascular clotting causing hypofibrinogenemia occurs after intravascular infusion of thromboplastin The latter is found in great amounts in decidual tissue and will pass the basal plate in abruptio placentae and in patients carrying a macerated stillborn fetus

The lowered fibrinogen level occurring after intravenous administration of pitocin may be of clinical significance in cases where a subclinical hypofibrinogenemia is found As stated previously this condition is encountered in abruptio placentae and a macerated stillborn fetus Intravenous pitocin administration may cause further lowering of the already depressed fibrinogen level and change the subclinical dyscrasia into a manifest one Therefore unless a rapid delivery with intravenous pitocin can be expected pitocin should not be administered in these two conditions

REFERENCES

1 Gornall A G Bardawill C J and David M M Determination of serum proteins by means of biuret reaction J Biol Chem 177 751 766 1949
2 Margulis Luzadre Hodgkinson Fibrinolysis in labor and delivery Obst Gyn N Y 3 487 1954
3 Wu H and Ling S M Colorimetric determinations of proteins in plasma cerebrospinal fluid and urine Chin J Physiol 1 161 1927

THE EFFECT OF DIETARY PROTEIN INTAKE ON THE METABOLISM OF N^{15} LABELLED GLYCINE IN PREGNANT WOMEN*

JOSEPH SEITCHIK AND CARL ALPER

This presentation concerns itself with the metabolism of glycine in pregnant women This particular nonessential amino acid was chosen because it has both general and specific metabolic functions It is not only incorporated into the peptide chain of protein but also into the purine ring of nucleoproteins and the porphyrin ring of hemoglobin It was therefore considered an excellent amino acid to study in conditions of growth and rapid hematopoeisis

The structure of this investigation was relatively simple Four normal pregnant women at 28 to 31 weeks of gestation were admitted to the hospital for care as ambulatory in patients All 4 women received isocaloric

From the Divisions of Women and Biological Chemistry Hahnemann Medical College and Hospital Philadelphia Pa This project has been supported in part by a grant (A 625) from the National Institute of Arthritis and Metabolic Diseases U S Public Health Service

Table 1 Control Group

GROUP I (WU LING)						
FIBRINOGEN	A	B	C	D	E	F
mg %	303	—	322	314	316	320
%	100%	—	105 9%	103 6%	104 2%	105 6%

GROUP II						
FIBRINOGEN	A	B	C	D	E	F
mg %	130	510	151	167	—	—
%	100%	118 6%	101 8%	108 6%	—	—

per cent or an increase of 1 2 per cent The range in Specimen F was from 170 mg per cent to 110 mg per cent with an average of 320 mg per cent or an increase of 5 6 per cent

Group B In this group the specimens were taken before administration of pitocin at the time of the delivery, half an hour after delivery, and 4 hours postpartum The fibrinogen determinations were made according to the Gornall method The pre pitocin specimen showed a variation in fibrinogen of 350 mg per cent to 680 mg per cent with an average of 516 mg per cent

The second specimen taken at time of delivery varied from 280 mg per cent to 560 mg per cent with an average of 416 mg per cent or a decrease of 22 4 per cent from the initial average value

The third specimen varied from 170 mg per cent to 670 mg per cent with an average of 557 mg per cent or an increase of 2 per cent over the initial value The fourth specimen values ranged from 380 mg per cent to 780 mg per cent the average being 492 mg per cent or a decrease of 9 8 per cent

COMMENTS

The very small number of cases does not allow us to make any valid conclusion statistically However there is one outstanding fact worth mentioning In all patients who did not receive any posterior pituitary extract the fibrinogen levels were almost unchanged during labor delivery and immediate postpartum course If any there was a slight trend toward an increase ranging from 1 2 per cent to 18 6 per cent This increase is statistically insignificant since the technical error in fibrinogen determinations is from minus 10 to plus 10

Only in patients receiving pitocin did we notice a decrease in the average value of 22 4 per cent These patients had a shorter and more tumultuous

Table 2 Pitocin Group

FIBRINOGEN	A	B	C	D
mg %	516	416	557	492
%	100%	77 6%	102%	90 2%

Table 2 The Effect of Dietary Nitrogen Intake on the Excretion of N^{15}

PT NO	ORAL INTAKE		URINE OUTPUT		INTAKE LESS OUTPUT		URINE UREA+NH_4		URINE URIC ACID	
	N GM/D*	N^{15} GM†	N GM/D	N^{15} GM‡	N GM/D	N^{15} GM‡	N GM/D	N^{15} GM‡	N GM/D	N^{15} GM‡
1	5.7	0.681	5.0	0.222	+0.7	+0.459	3.6	0.156	159	2.5
2	12.4	0.510	8.5	0.223	+3.9	+0.287	7.0	0.219	150	1.7
3	19.4	0.510	12.9	0.260	+6.5	+0.280	9.9	0.201	161	1.3
4	19.4	0.510	15.5	0.279	+3.9	+0.261	12.7	0.230	203	1.2

*D = 24 hours
†gm of N^{15} ingested as glycine at start of study
‡the quantity of N^{15} retained or excreted over the 8 day study period

It must be remembered that this isotope data cannot be interpreted on the same basis as the chemical data. The chemical values denote *metabolic balances* by quantitating intake and output, whereas the isotope studies reveal *rates* of metabolic processes within the balance. For example, the large retention of isotopic nitrogen observed in patient Number 1 over the 8 day study period does *not* signify that more protein is being synthesized but rather that this nitrogen has entered a protein pool in which the turnover rate is reduced. If we were to collect urine from all of these patients for a sufficient length of time and their feces as well, practically 100 per cent of the N^{15} would be recovered. But we would recover this material from patient Number 1 at a much slower rate than from patients Number 2 to Number 4.

It is inferred then that a dietary intake of 5.7 gm. of nitrogen per day is inadequate, even though the caloric intake is sufficient. This nitrogen intake has been considered inadequate in the past because we have presumed that growth of mother and fetus demands a significantly positive nitrogen balance. This conclusion is fortified by the demonstration that this low nitrogen intake results in a reduced turnover rate of nitrogen. The high protein (120 gm.) intake did not seem to enhance the rate of excretion of N^{15} when compared with the 80 gm. protein intake at a caloric intake of 2600 calories.

The content of excreted nitrogen in the urea+ammonia and uric acid fractions of the urine is reported in Table 2. The higher the nitrogen content of the urine, the greater was the quantity of the urea+ammonia fraction. This is to be expected in the patients fed the 80 and 120 gm. diets because calories of protein origin have been substituted for calories of carbohydrate and fat origin. In contrast, the urinary excretion of uric acid seemed relatively unaffected by these diets. Patient Number 4 excreted the most urate. Patient Number 3, who was fed the same high protein diet ingested by patient Number 4, excreted the same quantity of uric acid as patient Number 1 who received the least protein.

The excretion of isotopic nitrogen in the urea+ammonia and uric acid components of the urine is *different than that* of the non isotopic material. The patients on 80 and 120 gm. protein diets excreted the same quantity of isotope during the 8 day study period. The patient on the low protein diet

(2 600 calories), low purine diets that were varied in their protein content Patient Number 1 received 36 gm of protein per day patient Number 2 78 gm of protein per day and patients Number 3 and Number 4, 121 gm of protein per day By our present clinical standards these are low average and high protein diets respectively

After 3 to 5 days on these diets the patients received intravenously 150 mg of uric acid containing N^{15} (8 0 atoms per cent excess) Urine samples were collected every 4 hours for 1½ days and analysed for uric acid and isotopic nitrogen When the patients had been on the diet for 9 to 11 days they received labelled glycine (N^{15} content 62 atoms per cent excess) orally in a dose of approximately 80 to 100 mg /kg of body weight The glycine nitrogen represented a small additional quantity added to her regular dietary regimen For this and the next 7 days 24 hour urine specimens were collected and analysed for total nitrogen, urea and ammonia (as a single fraction) and uric acid The isotope concentration of each fraction was also determined

RESULTS

The data obtained following administration of the labelled uric acid are included in Table 1 They demonstrate that the turnover rates miscible pools, and turnover numbers were not affected by the dietary protein content It is possible that a different result might have been obtained had the patients been on their dietary routines for a longer period of time However, after dietary preparation of only 3 days duration no differences were observed in the rate of production of urate

The results obtained from the chemical and isotopic analyses following the administration of the glycine are listed in Table 2 As expected with increased dietary nitrogen intake there was increased urinary nitrogen output The difference between oral intake and urinary output was nominal in patient Number 1, and significantly large in patients Number 2 to Number 4 inclusive Patient Number 1 is considered in relative negative nitrogen balance because the normal state of nitrogen balance in pregnancy is the retention of more than 1 gm of nitrogen per day The remaining patients manifest significant nitrogen retention

The isotope data present a different pattern Patient Number 1, on the lowest protein intake and who received the largest dose of glycine retained the most N^{15} during the 8 day study period

Table 1 The Effect of Dietary Nitrogen on the Metabolism of Uric Acid

PT NO	I_0*	TURNOVER RATE	MISCIBLE POOL	TURNOVER NUMBER	%† RECOVERED
		POOLS/DAY	GM	GM /DAY	
1	1 26	1 06	0 85	0 91	55 8
2	1 35	1 05	0 74	0 78	71.5
3	1 36	1 20	0 72	0 86	63 0
4	1 41	1 04	0 70	0 73	81 7

*I_0 is the value obtained for the concentration of N^{15} labelled uric acid in the miscible pool immediately after injection and mixing

†Per cent of injected N^{15} recovered in the urine in 4 days

SUMMARY

Data was obtained on the excretion of N and N[15] in 4 normal pregnant women receiving isocaloric low purine diets of varying protein content. The low protein diet (36 gm./day) induced an enhanced rate of conversion of glycine to uric acid and a decreased rate of conversion of glycine to urea. This data is interpreted to suggest that this low protein diet is inadequate and that this alteration in the rate of conversion of glycine to urate is an attempt to maintain the tissue nucleoprotein mass in the face of nitrogen deficit. Whether this attempt at compensation is adequate and of what importance is the total caloric intake in this phenomenon we do not know. These and other questions demand the study of more patients at varying levels of protein and caloric intake.

CARDIAC OUTPUT DURING LABOR*

Charles H. Hendricks and Edward J. Quilligan

The changes in cardiac output in pregnancy have been extensively studied; however, to date very little data is available on changes in labor and the immediate postpartum period. To study adequately this period a determination of cardiac output is needed in which rapid change can be observed. The pulse pressure method of determining cardiac output seemed best for this purpose. This method was originally introduced by Erlanger and Hooker[1] in 1904 and has been modified recently by Remington *et al.*[5] Using the modification of Remington *et al.* we were able to obtain good correlation between pulse pressure and Evans blue cardiac output determinations in healthy young women.[3]

METHOD

The present study was performed on 47 healthy young women who were either in late pregnancy, in labor, or immediately postpartum. A number 18 Cournand needle was inserted into the brachial artery under local anesthesia and connected to a Sanborn Electromanometer. Pressures were recorded on a direct writing viso cardiette. Uterine contractions were recorded by a multiple lead externally recording tokodynamometer.

RESULTS

During the first stage of labor with a normal uterine contraction there was a rise in cardiac output which averaged 30.9 per cent. The increased output roughly paralleled in time the increase in uterine tone. One possible explanation for the rise in output would be the squeezing out into the systemic circulation of significant amounts of uterine blood with the onset of a uterine contraction.

Ineffective uterine contractions as observed in false labor or in so-called

* From the Department of Obstetrics and Gynecology, Western Reserve University School of Medicine, Cleveland, Ohio. This investigation was supported in part by a research grant (H-1914) from the National Heart Institute of the National Institutes of Health, Public Health Service.

excreted a smaller amount of N^{15}. This reduced excretion of isotopic nitrogen is also apparent in the urea+ammonia fraction of patient Number 1. On the other hand, the quantity of N^{15} recovered in the urinary uric acid is greatest in patient Number 1 who received the least protein. This finding indicates that the rate of incorporation of glycine into uric acid is increased in this patient on the low protein diet. The low isotope content of the urea+ammonia fraction and the high isotope content of the uric acid is interpreted to signify that the patient is attempting to maintain the rate of purine metabolism while the rate of conversion of glycine to urea, the major metabolic pathway, is reduced. This suggests that the pregnant human female has the capacity to alter her metabolic rates so that purine and presumably nucleoprotein metabolism may be maintained in the face of nitrogen deficit when the diet fed is calorically adequate.

If the data for urinary N and N^{15} is expressed as a percentage of the dietary N or N^{15} (Table 3) the inferences are unchanged. Patient Number 1, who was on the low protein diet, excreted the largest portion of dietary nitrogen and excreted the smallest share of fed N^{15}. She also excreted the smallest percentage of N^{15} in the urea+ammonia fraction of urinary nitrogen and excreted the maximal percentage of both N and N^{15} in the uric acid.

If these data are calculated in terms of the distribution of N and N^{15} in the excreted nitrogen (Table 4) we see that the altered diets have not produced gross changes in the percentage of N and N^{15} in the urea+ammonia fraction of the urinary nitrogen, although the patient on the low protein diet has the smallest value. In contrast, both the percentage of N and N^{15} in the uric acid is increased markedly by the low protein diet.

Table 3. Per Cent of Fed N or N^{15} Excreted in Urine as

PT NO	TOTAL N	TOTAL N^{15}*	UREA+NH_3 N	UREA+NH_3 N^{15}	URIC ACID N	URIC ACID N^{15}*
1	87.7	32.6	63.2	22.0	2.8	0.37
2	68.6	16.9	56.5	10.6	1.2	0.31
3	66.5	18.1	51.1	37.2	0.9	0.21
4	80.1	51.7	65.5	12.6	1.0	0.2[illegible]

* The per cent of N^{15} recovered in the 8 day study period

Table 4. Per Cent of Urine N or N^{15} Excreted as

PT NO	UREA+NH_3 N	UREA+NH_3 N^{15}	URIC ACID N	URIC ACID N^{15}
1	72.0	70.2	3.2	1.10
2	82.3	86.[illegible]	1.8	0.66
3	76.8	77.3	1.3	0.50
4	82.0	82.5	1.3	0.15

* In the 8 day study period

ANTAGONISM OF GLUCOCORTICOIDS AND ACTH ON ESTRADIOL 17β INDUCED UTERINE GROWTH*

JOSEPH T VELARDO AND SOMERS H STURGIS

Recent studies have emphasized that interactions among hormones of the adenohypophysis suprarenal cortex and the ovary, may modify certain responses of the female reproductive system [1 2]

The present study records one such interaction between hydrocortisone acetate (Compound F) or 9 αFluoro-hydrocortisone acetate (Fluoro F) and estradiol 17β We have used the uterus of the ovariectomized rat as a test organ In an effort to elucidate the mechanisms involved we have also used a highly purified ACTH preparation in animals after removing the adrenals as well as the ovaries

METHOD

Virgin albino rats 100 days of age and weighing approximately 200 gm were ovariectomized bilaterally and started on the experiment 7 days later Estradiol 17β was dissolved in sesame oil and Compound F and 9αFluoro F were dissolved in an aqueous vehicle containing 0 9 per cent benzyl alcohol 3 drops of tween 60 and physiological saline solution The ACTH used in these experiments was ACTHAR GEL All injections were administered once daily subcutaneously When 2 substances were administered they were injected at separate sites Necropsies were performed 72 hours after the initial injection and the uteri and adrenals were weighed on a Roller Smith torsion balance Twenty four hours later the dry solid content of the test organs was determined

RESULTS

Controls It was established previously[6] that both the vehicles used for the hormones were without effect in modifying the response of the uterus to estradiol 17β

Uterine growth Promoting Effects of Estradiol 17β Hisaw Velardo and Goolsby[7] using identical procedures as described above found that 0 1 μg estradiol 17β given daily for 3 days would produce the steepest rise in uterine growth on their dose response curve This treatment instituted 1 week after ovariectomy produced uteri that were 88 per cent heavier than the uteri from non treated ovariectomized controls In the present experiments with rats from the Charles River strain rather than those of the Harvard Biological Laboratory strain previously utilized[7] a comparable increase in uterine weight was observed Consequently 0 1 μg estradiol 17β was chosen as the standard dosage of estrogen

The term growth as used in this report is based entirely on dry uterine weight determinations On previous occasions nitrogen assays were determined on each group of uteri from the animals on each treatment schedule and it was found that the results of the nitrogen determinations follow precisely the curves for the dry weights

*From the Departments of Gynecology and Surgery Harvard Medical School and Peter Bent Brigham Hospital Boston Massachusetts Supported in part by a grant from the United States Public Health Service C 2546 National Institutes of Health The technical assistance of Miss Nancy M Raney and Miss M Claire Simoneau is gratefully acknowledged

pitocin contractions resulted in cardiac output rises averaging only 18 per cent above the baseline levels Furthermore this rise in cardiac output did not necessarily parallel the observed increase in uterine tone

The act of 'bearing down in nonpregnant controls and in pregnant patients who were not having uterine contractions causes a temporary drop in cardiac output followed by a rise after exhalation Such a result might have been predicted from the work on arterial pressures previously reported by Gorlin *et al*[2] However, the act of bearing down with a uterine contraction during labor was associated with changes in cardiac output which were highly unpredictable It is assumed that the great variability of the output pattern when the Valsalva maneuver was performed during uterine contraction is brought about to a large extent by the rapid fluctuations in the central venous reservoir occurring after the onset of a contraction It is of further interest to note that arterial tracings of some patients who performed the maneuver in the early phase of uterine contraction were surprisingly similar to tracings observed by others[4] who were studying patients with early pulmonary congestion

It has been postulated that the cardiac output rises postpartum are due in large part to the cessation of the maternal portion of the placental circulation, which has been considered to function in a manner somewhat similar to an arteriovenous fistula An attempt was made to demonstrate such an effect In 5 cases of cesarean section and in 3 vaginal deliveries manual removal of the placenta was carried out Contrary to the expectation, it was found that of the 8 cases only 2 showed a significant rise immediately upon the removal of the placenta (11 per cent and 20 per cent, respectively) while in the remaining 6 cases the immediate output following placental removal remained essentially the same or dropped In all 8 cases however the output increased in some degree between ½ minute and 2 minutes after removal of the placenta the increase ranging from 1 to 41 per cent and averaging 11 per cent but within 10 to 20 minutes the outputs had returned in most cases to lower values usually at levels lower than those existing prior to the removal of the placenta

SUMMARY

1 Cardiac output increases with an effective uterine contraction by an average of 30 9 per cent

2 During the second stage of labor the changes in cardiac output are variable

3 There is usually a slight rise in cardiac output postpartum which could not be demonstrated to be due to cessation of maternal placental circulation

REFERENCES

1 Erlanger J and Hooker D R An experimental study of blood pressure and of pulse pressure in man Bull Johns Hopkins Hosp *12* 145 1904

2 Gorlin R Knowles J H and Storey C F The detection of pulmonary congestion utilizing the Valsalva maneuver J Clin Invest *34* 936 1955

3 Hendricks C H and Quilligan E J Cardiac output in pregnancy correlation between Evans blue dye and blood pressure methods Circul Res N Y (in press)

4 Judson W E Hatcher J D and Wilkins R W Blood pressure responses to the Valsalva maneuver in cardiac patients with and without failure Circulation N Y *11* 889 1955

5 Remington J W Noback C R Hamilton W F and Gold J J Volume elasticity characteristics of human aorta and prediction of stroke volume from pulse pressure Am J Physiol *153* 298 1948

ANTAGONISM OF GLUCOCORTICOIDS AND ACTH ON ESTRADIOL 17β INDUCED UTERINE GROWTH*

Joseph T Velardo and Somers H Sturgis

Recent studies have emphasized that interactions among hormones of the adenohypophysis suprarenal cortex and the ovary, may modify certain responses of the female reproductive system[1, 2]

The present study records one such interaction between hydrocortisone acetate (Compound F) or 9 αFluoro-hydrocortisone acetate (Fluoro F) and estradiol 17β We have used the uterus of the ovariectomized rat as a test organ In an effort to elucidate the mechanisms involved we have also used a highly purified ACTH preparation in animals after removing the adrenals as well as the ovaries

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Experimental Data 1 *Influence of Hydrocortisone Acetate on Estradiol induced uterine growth* Compound F acetate in daily doses of 0 01 to 1 25 mg does not seem to alter appreciably the growth potential of the uterus (Fig 1) Additional groups of animals on dosages between 1 25 and 5 0 mg showed no significant departure from the arithmetical mean of the non treated controls

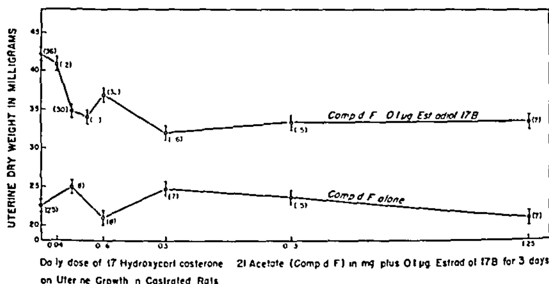


Fig 1 The response of the uteri of ovariectomized rats to 17 hydroxycorticosterone-21 acetate (Compound F) and a combination of Compound F plus 0 1 µg estradiol 17β (The numbers in parentheses indicate the number of animals on each point)

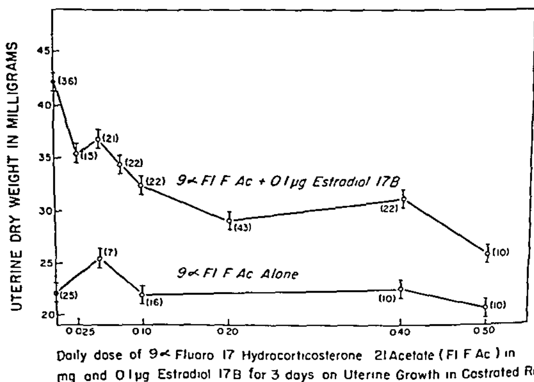


Fig 2 The effect of 9 Fluoro-17 hydroxycorticosterone 21 acetate on the uteri of ovariectomized rats with and without 0 1 µg estradiol 17β

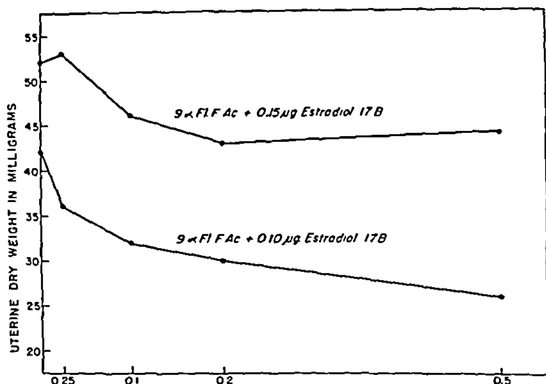


Fig 3 The effect of increasing the dose of estradiol 17β from 0 1 μg to 0 15 μg given in combination with 9 Fluoro 17 hydroxycorticosterone 21 acetate

In the groups of animals receiving a combination of Compound F and estradiol 17β an antagonism to the uterine growth promoting effects of the estrogen could be observed with as little as 0 08 mg of this adrenal steroid No further restriction of uterine growth occurred when more than 0 32 mg of Compound F were administered

2 *Action of 9 αFluoro hydrocortisone Acetate on Estradiol Induced Uterine Growth* When administered alone 9 αFluoro-F acetate had no significant effect on the dry weights of the uteri of ovariectomized rats On the other hand it is obvious from Figure 2 that Fluoro-F antagonizes the normal uterine growth response to this dose of estradiol A dramatic decrease can be obtained with as little as 0 025 mg While the antagonism of estradiol by this hormone seems constant at the 0 2 to 0 4 mg level a dose of 0 5 mg further reduces the ability of the uterus to respond to the standard dose of this estrogen Any such antagonistic interactions are clearly a function of dosage of hormones used We chose 0 1 μg estradiol because from previous work this appeared to act physiologically on the uterus of the castrate rat Higher and unphysiologic doses can produce a greater end organ response Thus when 0 15 μg estradiol is given the uterine dry weight is increased approximately 25 per cent If the same dose range of Fluoro F acetate is combined with this increase in estrogen there is seen little significant suppression of growth The uterine weight is unchanged at the smallest dose of the adrenal corticoid and at the 0 5 mg level there was only a 16 per cent instead of a 39 per cent inhibition

of growth This data illustrates the precise nature of response of interactions between hormones In this case the stimulation of a larger dose of estrogen is capable of virtually wiping out the inhibitory action of the corticord

3 *Influence of Purified ACTH on Estradiol Induced Uterine Growth* Since Compound F and Fluoro-F were both effective in antagonizing the action of estradiol 17β on uterine growth it seemed appropriate to determine the effects of ACTH in similarly prepared animals The data clearly indicate that when as little as 0 025 mg of ACTH was administered concurrently with 0 1 μg estradiol 17β the growth response was significantly reduced It appears that 0 10 mg ACTH effects the greatest decrease in the growth of the uterus.

It is noteworthy that the adrenal glands of the animals receiving ACTH all demonstrated a definite increase The adrenals in the ACTH-estradiol treated rats were at least 10 mg heavier than those of the estrogen treated group

To ascertain the pathway by which ACTH inhibited the action of estradiol 17β in these experiments bilateral adrenalectomy was performed one hour before the administration of the hormones in previously ovariectomized rats All other procedures were adhered to precisely as described previously In Figure 3 it is seen that when 0.5 mg ACTH was administered with 0 1 μg estradiol 17β the response of the uterus was markedly decreased When the same substances were given to animals that were

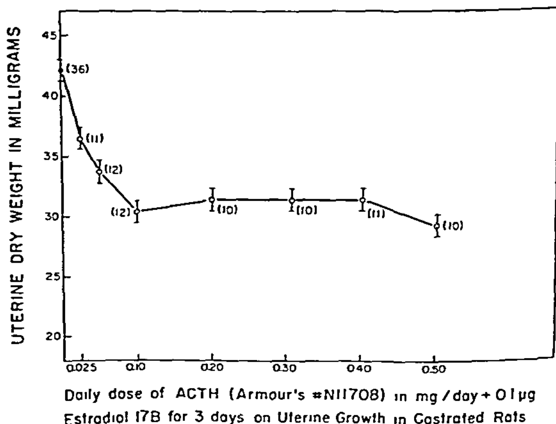


Fig 4 The restrictive influence of ACTH on estradiol 17β induced uterine growth.

Table I Mechanism of ACTH Inhibition of Estradiol 17β Induced Uterine Growth in Ovariectomized, Albino Rats

TREATMENT	NO OF RATS	UTERINE WET WEIGHT	UTERINE DRY WEIGHT
Ovariectomized controls without any treatment	25	1155 ±33	22.4 ±065
Ovariectomized 0 1 µg Estradiol 17B	36	2273 ±6 1	42 3 ±1 8
Ovariectomized 0 1 µg Estradiol 17B + 0.5 mg ACTH	10	156 9 ±7 9	29 2 ±1 3
Ovariectomized and Adrenalectomized 0 1 ug Estradiol 17B	7	249 7 ±13 2	45 1 ±2 4
Ovariectomized and Adrenalectomized 0 1 µg Estradiol 17B + 0 5 mg ACTH	10	251 7 ±13 5	47 0 ±2 3

bilaterally adrenalectomized as well as ovariectomized however the response of the uterus was not altered

It seems justifiable to conclude therefore that the mechanism of the ACTH inhibition of estrogen induced uterine growth is mediated by the adrenal glands

DISCUSSION

By the use of ovariectomized rats on a standard regime of estrogen stimulation the growth response of the uterus can be standardized Hydrocortisone acetate effectively reduces this response The halogenation in position 9 of this compound causes an even greater antagonizing effect on the growth of the uterus ACTH also inhibits the induction of uterine growth That this is not a direct action of ACTH on the uterus itself but is mediated through the adrenal glands is shown by the failure of this effect from ACTH in animals on estradiol 17β that were both adrenalectomized and ovariectomized

The data here presented give added confirmation to the work of Szego and Roberts[8] and Szego[9] who showed that the uterine imbibition of water caused by estrogen could be suppressed by Compound F and ACTH using a 4 hour intravenous technique We have found that the growth response as well indicated by dry weights is also inhibited This mechanism is controlled in a precise way by the balance or ratio of hormones involved as demonstrated by these experiments

It is exceedingly difficult to define with clarity the complex hormonal interactions that contribute in the formation of certain recognized clinical syndromes This study calls attention to functional pathways whereby ACTH reacts in antagonism to estrogen on the uterus of the ovariectomized rat.

CONCLUSIONS

These data show conclusively that hydrocortisone acetate 9 αFluorohydrocortisone acetate, and ACTH interfere with the action of estradiol 17 β in the promotion of uterine growth in ovariectomized rats The mechanism of ACTH inhibition of estradiol 17 β induced uterine growth was found to be mediated by the adrenal glands These data lend credence to the concept that hormones and/or their metabolites work *in concert* to produce the end organ response

REFERENCES

1 Benson R C Kolb F O and Traut H F Hirsutism defeminization and virilization The endocrine basis for treatment Obst Gyn N Y *5* 307 319 1955
2 Abu Hydar N Laidlaw J C Nusimovich B and Sturgis S H Hyperadrenocorticism and the Stein Leventhal syndrome J Clin Endocr Metab *14* 766 1954
3 Velardo J T, Hisaw F L and Beyer A T Inhibition of estradiol 17β induced uterine growth in rats by desoxycortisone acetate testosterone and cortisone acetate Anat Rec *117* 552 1953
4 Szego C M and Roberts S Steroid action and interaction in uterine metabolism Recent progress in hormone research Vol 8 Pincus G ed New York Academic Press 1953 pp 419-469
5 Roberts S and Szego C M Steroid interaction in the metabolism of the reproductive target organs Physiol Rev *33* 593 629 1953
6 Velardo J T Inability of several vehicles and pregnane 3α 20α diol to modify the response of the uterus to estradiol 17β In Press.
7 Hisaw F L Velardo J T, and Goolsby C M Interactions of estrogens on uterine growth J Clin Endocr Metab *14* 1134 1145 1954
8 Szego C M, and Roberts S Pituitary adrenal cortical antagonism to estrogenic stimulation of the uterus of the ovariectomized rat Am J Physiol *152* 131 140 1948
9 Szego C M Pituitary adrenal cortical antagonism to estrogenic stimulation of the uterus of the ovariectomized rat Observations on structural specificity of crystalline steroids Endocrinology *50* 429 441 1952

INSULIN TOLERANCE IN PREGNANCY*

RICHARD L BURT

METHOD

In an attempt to characterize the changes imposed by pregnancy on carbohydrate metabolism and to supplement previous studies from this laboratory concerning the utilization of glucose insulin tolerance determinations were made on 20 patients between the eighth and twenty sixth week of gestation as well as an additional group of 20 between the thirty sixth week and term An equal number of postpartum patients were studied on the fourth or fifth postpartum day Twenty normal nonpregnant subjects served as controls

Regular insulin 0 1 unit/kg body weight was administered intravenously after obtaining a pretest blood specimen The blood sugar con

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centration was then followed at intervals for one hour. Parallel determinations were made of the plasma inorganic phosphorus. The methods used have been described in a previous report.[1]

In all subjects studied maximum depression for blood sugar concentration occurred at the 20 minute interval following insulin administration. Between the eighth and twenty sixth week of gestation the responses were identical to those of the control subjects. In late pregnancy (36 to 40 weeks) the slope of the dose response curves tended to be less than found for early pregnancy or control subjects. The mean minimum blood glucose value for this group was 57.6 mg per cent ($\theta = 13.1$ mg per cent) at 20 minutes in contrast to 81.6 mg per cent ($\theta = \pm 9.5$ mg per cent) found prior to 26 weeks. During the early puerperium the blood glucose data resembled that of late pregnancy but there appears to be a definite tendency toward return to normal nonpregnant responses. In Figure 1 the mean values of glucose are plotted for each category of subjects studied.

The plasma inorganic phosphorus concentration was depressed to a maximum approximately 10 minutes after insulin administration in all groups. The mean decrease in phosphorus concentration for pregnant and postpartum subjects amounted to 0.6 and 0.7 mg per cent respectively in contrast to the value found for control subjects of 1.3 mg per cent.

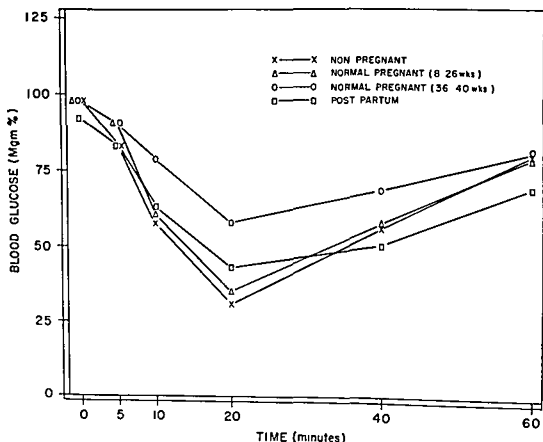


Fig. 1. Mean blood glucose values following 0.1 unit regular insulin/kg body weight in nonpregnant, pregnant and postpartum subjects.

COMMENT

One aspect of carbohydrate metabolism in late pregnancy appears to be the development of increased resistance to insulin which has been demonstrated between the thirty sixth and fortieth week. In the majority of patients approaching term the attenuation of blood glucose responses was definite and contrasted sharply with a marked and consistent response found for nonpregnant subjects. Prior to 26 weeks no obvious nor apparent statistical difference was observed in insulin tolerance when compared to the controls. Serial determination of tolerance was not performed so that individual variance in insulin sensitivity cannot be assessed at the present time and the exact time relationships of changes in tolerance throughout pregnancy and the puerperium are not described by this data.

The decreased response of plasma inorganic phosphorus to insulin administration during pregnancy and the early puerperium is difficult to interpret. A similar decreased response was observed in normal gestation following glucose administration. Although an abundant literature exists concerning the relation of insulin to phosphate metabolism[2] data are lacking that indicate specific gestational changes in oxidative phosphorylation reactions which could account for such differences in phosphate response.

The data presented may be related to the clinical course of diabetes in pregnancy. In terms of insulin requirement the pregnant patient tends to exhibit an increase in the severity of the metabolic error requiring increasing amounts of insulin to maintain regulation. This increased severity of the diabetic appears to be transient with decrease to antepartum insulin requirements following delivery.

It is probable that total metabolism of carbohydrate in gestation is influenced by many factors and the precise significance of the reported change in insulin sensitivity is difficult to assess. However this normal loss of insulin sensitivity is consistent with the clinical course of diabetes in pregnancy and with the clinical observation that insulin requirement *is decreased following delivery*.

From a large body of experimental literature and clinical observation it is apparent that blood glucose concentration and insulin reactivity may reflect changes in pituitary or adrenal function.[3] Alterations in insulin sensitivity in human subjects or experimental animals may be indicative of deviations in this hormonal balance and loss of insulin sensitivity in late pregnancy may be based on pituitary or adrenal cortical factors. Details of such a mechanism are not known at the present time but this view is consistent with cytological studies of the hypophysis and adrenal cortex of pregnancy as well as biochemical observations concerning the steroids and gestation. Although the described gestational decrease in reactivity to insulin may be demonstrated experimentally homeostasis is normally so perfect that significant alterations in fasting blood sugar and glucose tolerance are not observed.

SUMMARY

1. Insulin sensitivity is decreased in normal human pregnancy after 36 weeks. Prior to 36 weeks no change in insulin tolerance was found.

2 The data suggest a tendency to reversion toward normal nonpregnant responses by the fourth or fifth postpartum day

REFERENCES

1 Burt R L Peripheral utilization of glucose in pregnancy and the puerperium Obst Gynec., N Y 4 58 1954
2 Stadie W C Current concepts of the action of insulin Physiol Rev 34 52 1954
3 Ciba Foundation Colloquia on Endocrinology Vol VI Hormonal factors in carbohydrate metabolism Boston Little Brown and Company 1953

AN EXPERIMENTAL AND CLINICAL REEVALUATION OF CHLOROFORM ANESTHESIA IN OBSTETRICS*

Paul T Buerger George M Sanderson, Jr and Albert C Rekate

The ideal obstetrical anesthetic one which is completely safe for both mother and baby and which gives complete relief of pain in all cases has not yet been discovered For this reason there is tremendous divergence of opinion in medical circles as to which of the available anesthetic agents is best suited for use in obstetrics In Buffalo we have used chloroform anesthesia for the past 50 years with great satisfaction

Advantages of Chloroform Anesthesia *Ease of Administration* Chloroform is supplied by the manufacturer in 20 cc bottles with dropper tops and the only additional equipment necessary is an ether mask covered with 8 layers of gauze

Pain Relief Chloroform is a general anesthetic and therefore offers complete relief of pain

Relaxation Complete relaxation is obtained for any obstetrical maneuver which may be necessary including rotations breech extractions and versions

Induction Surgical plane anesthesia is obtained in 5 minutes and the excitement phase is short

Recovery Recovery from anesthesia is rapid and although vomiting is not infrequent aspiration is uncommon no deaths occurring from aspiration in our series of 209 000 cases

Depression of Fetal Respirations Respirations were spontaneous in 91 per cent of 60 000 chloroform anesthetics studied by Lenahan and Babbage [1]

Maternal Anesthetic Mortality For many generations the toxicity of chloroform in regard to both the heart and liver has been greatly stressed in medical teaching No one will deny that chloroform is a potentially dangerous drug but when used with care and understanding the actual anesthetic risk is less than half that of other commonly used obstetrical anesthetics It is with this particular phase of chloroform anesthesia with which this paper is primarily concerned

*From the Department of Obstetrics and Gynecology Edward J Meyer Memorial Hospital Buffalo New York

COMMENT

One aspect of carbohydrate metabolism in late pregnancy appears to be the development of increased resistance to insulin which has been demonstrated between the thirty sixth and fortieth week In the majority of patients approaching term the attenuation of blood glucose responses was definite and contrasted sharply with a marked and consistent response found for nonpregnant subjects Prior to 26 weeks no obvious nor apparent statistical difference was observed in insulin tolerance when compared to the controls Serial determination of tolerance was not performed so that individual variance in insulin sensitivity cannot be assessed at the present time and the exact time relationships of changes in tolerance throughout pregnancy and the puerperium are not described by this data

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SUMMARY

1 Insulin sensitivity is decreased in normal human pregnancy after 36 weeks Prior to 36 weeks no change in insulin tolerance was found

system The primary effect however is upon the ventricles producing ectopic foci in varying degrees of severity Ventricular tachycardia was found in one third of the cases and other minor abnormalities were found in an additional 30 to 50 per cent of the cases depending on whether supplementary oxygen was given under the mask All the observed disturbances reverted spontaneously to a normal rhythm at or before the termination of the anesthetic In the past it had been thought that cardiac irregularities occurred during induction with chloroform anesthesia or secondary to painful stimuli under light anesthesia In this series however we find that the development of ectopic rhythms follow no set pattern and are as likely to occur in the recovery phase as at any other time during the anesthesia or delivery The attempt to improve oxygenation resulted in an overall decrease in the number of irregularities but did not alter the frequency of severe irregularities namely ventricular tachycardia Pronestyl was tried in a few cases but failed to either prevent or break an abnormal rhythm in doses which were considered safe for the mother and baby

Review of 209,000 Chloroform Anesthetics Twenty thousand cases of chloroform anesthesia given over the past 5 years at the Buffalo Sisters of Charity Hospital were recently reviewed by one of the authors In this series of 20 000 cases there was 1 death which was due to cardiac arrest In addition there were 3 cases of aspiration 1 case of pneumonitis 2 cases of toxic hepatitis 1 case of auricular fibrillation and 1 case of paroxysmal tachycardia all of which recovered spontaneously In an unreported series of anesthetic deaths from the files of the Erie County Maternal Mortality Committee covering a 10 year period for 1933 to 1945, Dr Robert McDowell found 6 deaths due to chloroform anesthesia in 129 530 cases which had received chloroform anesthesia for an incidence of 1 in 21 588 Five of these deaths were due to cardiac arrest and one to delayed chloroform poisoning Two Buffalo anesthetists Dr Rose Lenahan and Dr Dean Babbage reviewed 60 000 chloroform anesthetics from 1916 to 1950 and found 4 deaths for an incidence of 1 in 15 000 If these 3 series are totaled together there were 11 chloroform deaths in 209 530 cases for an incidence of 1 in 19 000

Comparison With Other Anesthetic Agents Halperin and Levine[2] reported on 21 617 conduction anesthetics In this series they had 5 deaths for an incidence of 1 in 4 300 Fitzgerald and Webster[3] reported on 104 000 obstetrical anesthetics with 13 deaths for an incidence of 1 in 8 000 In the series from the Erie County Maternal Mortality Committee referred to previously there were 6 818 anesthetics including nitrous oxide spinal cyclopropane and ether In this group there were 6 deaths for an incidence of 1 in 1 100

CONCLUSION

We feel that the dangers of chloroform anesthesia have been unjustly exaggerated in the past In our series of 209 000 cases we have found that chloroform carries less than half the risk of other commonly used anesthetic agents We highly recommend it to anyone looking for an obstetrical anesthetic which offers ease of administration safety for the mother and baby complete pain relief and unexcelled relaxation for all obstetrical maneuvers

Precautions *Medical Contraindications* Chloroform should be avoided in cases of dehydration diabetes cardiac and liver disease

Preparation Adequate pre medication and slow induction greatly lowers the incidence of apnea in the induction phase

Administration Chloroform should be given slowly The level of anesthesia should be maintained just below the threshold of stimulation to avoid overdose There is no danger of stimulation under light anesthesia causing an increase in the incidence of cardiac irregularities

Amount The total dose should be limited to 20 cc This amount is usually sufficient for 25 to 10 minutes of anesthesia The amount has no effect on the development of cardiac irregularities but the incidence of liver involvement sharply increases if this amount is exceeded

Adequate Oxygenation A free airway must be maintained and two fingers should be kept under the mask or supplementary oxygen given by catheter

Additional Equipment A suction machine should be readily available to prevent aspiration of vomitus under anesthesia It is also highly desirable to have a gas machine available to give straight oxygen under pressure in cases of breath holding laryngospasm and accidental overdose

Because of the concern expressed in the standard pharmacology texts in regard to the cardiac and liver toxicity of chloroform we have under taken the following experimental and clinical study to determine the anesthetic risk of chloroform as compared to other commonly used anes thetic agents The method of study was (1) to determine in incidence and type of cardiac irregularities by continuous electrocardiographic mon itoring during 100 chloroform anesthetics and (2) to determine the clinical significance of these irregularities in a series of over 209,000 chloroform anesthetics given in Buffalo over the past 20 years

Electrocardiogram Series Under Chloroform Anesthesia *Series* Three groups of 50 cases each The first group of 50 cases was the control group and consisted of 20 cases of open drop ether 10 cases of low spinal 10 cases of pudendal block and 10 cases which delivered spontaneously without anesthesia The second group consisted of 50 cases of open drop chloroform anesthesia The third group consisted of an additional 50 cases of chloro form anesthesia in which an attempt was made to improve the oxygenation by administering oxygen under the mask at the rate of 4 liters per minute The limitations on this method of attempting to increase the oxygen saturation of the blood are readily apparent because we still have the problem of laryngospasm and the frequent periods of apnea experienced during induction with chloroform anesthesia to deal with

Technique In the second stage of labor the patient was taken to the delivery room and a pre anesthetic electrocardiogram of the 6 standard leads was then taken to rule out any unsuspected pre existing cardiac lesions The electrical activity of the heart was then monitored throughout the anesthetic by means of an oscilloscope which was attached in series with the direct writing electrocardiogram machine Whenever an abnor mality was noted on the oscilloscope screen a tracing was made for a permanent record and later detailed study by a cardiologist

Results Chloroform affects the heart as a whole including the auricles ventricles conduction system and possibly also the autonomic nervous

monthly and re-biopsied at 6 month intervals. Fifty three of these have been followed for as long as 3 years and none have become invasive while under our observation. In addition 18 cases of invasive squamous cell carcinoma were found. 4 patients had adenocarcinoma and 1 other malignant tumors were discovered. This gives a total of 56 malignant tumors or 0.67 per cent of all patients screened. Of these cancers 3 were reported on initial biopsy as carcinoma *in situ*, but an immediate histologic review of further tissue proved the neoplasm to be invasive. If further and immediate study had not been done these would have later been wrongly regarded as *in situ* cases which became invasive.

The age range in our series is from 17 to 77 years at time of diagnosis. The average age of patients in this series of carcinoma *in situ* is 42.9 years. That of Puerto Rican women with invasive cervix carcinoma is 49.2 years as opposed to the average age of 52.0 years for all female cancer patients in Puerto Rico.

DISCUSSION

From a strictly scientific viewpoint it has not yet been conclusively proved that carcinoma *in situ* is the pre-invasive stage of cervical cancer although it is a widely accepted and logical working hypothesis. Up to the present time only about 50 cases have been reported in which invasive cancer has been found subsequent to a previous finding of carcinoma *in situ*.[3] Otherwise only an occasional histologic study of this lesion has revealed what was interpreted as early invasion. Fennell[4] reported 8 such cases. The present follow up study was motivated by the possibility of observing such a transition clinically.

The possibility of also observing possible histochemical changes in the course of such a transition from carcinoma *in situ* to invasive carcinoma prompted a special study of 22 of these 53 cases all with previous histologic diagnoses of carcinoma *in situ*. Additional biopsy material from these 22 cases consisting of endometrial curettage, endocervical biopsy, biopsy from the portio and a vaginal biopsy were fixed in absolute alcohol and some of the material was fixed in neutral buffered formalin. Microscopically this additional tissue from 11 of these cases continued positive for carcinoma *in situ*. Glycogen stains, *PAS* stains and stains for alkaline phosphatase were made on the tissue from these 11 cases.

Glycogen and alkaline phosphatase were present in all but 1 of these 11 cases of carcinoma *in situ* although in decreased and varying amounts. It is possible that cytochemically diminishing stores of glycogen together with its related enzyme systems may indicate de-differentiation and therefore increasing malignancy. Foraker[5] in a study of 28 cases of squamous cell carcinoma has demonstrated such decreasing amounts of glycogen in the more undifferentiated areas of the cancers.

A study was also made of the cases of carcinoma *in situ* which were encountered in the course of a review of the histologic sections of 811 cases of carcinoma of the cervix which were accumulated in the files of the pathology department of the Wayne County General Hospital between 1932 and 1955. This study is being reported separately but the following is a summary of the results.

Sixteen cases of endocervical neoplasms were found in which the adjacent portio vaginalis was found to be the site of carcinoma *in situ*. In all 16

REFERENCES

1 Lenahan R M and Babbage E D A review of chloroform anesthesia in obstetrics N York State J M *50* 1717 1720 1950
2 Halpern J and Levine W Incidence of maternal mortality related to anesthesia Anesthésie Par *31* 301 308 1952
3 Fitzgerald J L and Webster A Nineteen year survey of maternal mortality at Cook County Hospital Am J Obst *65* 528 538 1953

THREE YEAR FOLLOW UP OF FIFTY THREE CASES OF CARCINOMA IN SITU OF THE UTERINE CERVIX*

LYNDON L LEE JR P J MELNICK AND HARRY M WALSH

This report on 53 cases of carcinoma *in situ* of the uterine cervix represents part of a 5 year program of clinical cytologic and histologic study and follow up of carcinoma of the cervix in a large and uniform population It was carried out by the senior author in Puerto Rico with the cooperation of the Puerto Rico Department of Health and Medical School, under financing by grants from the National Cancer Institute Over 8 500 women from the general population were screened by pelvic and cytologic examination All of the cases reported suspicious by these methods were biopsied A total of 67 cases of carcinoma *in situ* were found Fifty three of these cases were followed by repeated clinical cytologic and biopsy examinations up to 3 years without as yet evidence of invasion in any of them

RESULTS

In screening 8 500 patients by cytologic methods 38 per cent were found to have an abnormal smear Of these 9 out of 10 or 33 1 per cent of all cases had an abnormal cytologic pattern not regarded as cancer Reported abnormal patterns in this group resulted from infection high estrogen level atypical cells and smears unsatisfactory for interpretation due to excessive blood Cytologically 3 per cent of all cases were found to be suspicious of cancer 0 5 per cent were regarded as carcinoma *in situ* and 0 6 per cent were reported carcinoma

All of the suspicious cases were biopsied Five hundred and eighty four patients were subjected to one or more biopsies of the cervix Of these 461 revealed no malignancy Thus, combining clinical cytologic and histologic means of study 98 7 per cent of the total patients screened showed no malignancy

Various non malignant abnormalities (the benign hyperplasias) were encountered in the biopsy series These cases are being followed monthly and re biopsied at periodic intervals of not more than one year in order to determine their course

Sixty seven cases of carcinoma *in situ* were proved histologically a frequency of 0 79 per cent of the entire series They have been re smeared

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*From the Cancer Program of the Government of Puerto Rico and the Wayne County General Hospital Eloise Michigan This investigation was supported by a grant from the National Cancer Institute of the United States Public Health Service

monthly and re-biopsied at 6 month intervals. Fifty-three of these have been followed for as long as 3 years and none have become invasive while under our observation. In addition, 18 cases of invasive squamous cell carcinoma were found, 4 patients had adenocarcinoma, and 4 other malignant tumors were discovered. This gives a total of 56 malignant tumors or 0.67 per cent of all patients screened. Of these cancers 3 were reported on initial biopsy as carcinoma *in situ* but an immediate histologic review of further tissue proved the neoplasm to be invasive. If further and immediate study had not been done these would have later been wrongly regarded as *in situ* cases which became invasive.

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Sixteen cases of endocervical neoplasms were found in which the adjacent portio vaginalis was found to be the site of carcinoma *in situ*. In all 16

the associated carcinoma *in situ* was found separate from the endocervical neoplasm

These cases suggest that the occurrence of carcinoma *in situ* reflects a tendency of the involved cervix as a whole to develop neoplasms Only 1 such case has previously been reported[6]

This concept is further supported by the following additional cases that were encountered in the pathology department files 10 cases of carcinoma *in situ* adjacent to carcinoma of the portio whose histologic patterns were distinctly different and therefore not the result of invasion and spread along the surface by the carcinoma 6 cases of leukoplakia overlying large areas of carcinoma *in situ*, in 1 of which early invasion was found and 1 case in which ample glycogen was found in the carcinoma *in situ*, but very little in the adjacent Grade II squamous cell carcinoma A total of 33 neoplastic processes were therefore found in which an adjacent and separate carcinoma *in situ* was present

In an additional 38 cases carcinoma *in situ* was also found adjacent to the invasive carcinoma but was fused with it so that it was impossible to rule out spread along the surface epithelium by the invasive carcinoma For the purpose of this study it was therefore deemed essential to include only those cases in which the two were seen to be distinctly different

In an additional 20 cases of invasive cervical cancer atypical hyperplasia of the epithelium of the adjacent portio was observed but this histologic picture is not at the present time regarded as significant and much research remains to be done on this problem

In none of the above cases was there a record of therapy with antifolic acid substances which might induce epithelial atypism as reported by Weston and Guin[7]

No carcinomas *in situ* were found associated in 53 cases of carcinoma of the uterine fundus 2 cases of choriocarcinoma and 9 cases of leiomyosarcoma of the uterus that were reviewed

CONCLUSIONS

1 Fifty three cases of carcinoma *in situ* have been followed untreated for periods up to 3 years using monthly smears and biopsies at 6 month intervals So far there has been no evidence of invasive change Follow up is planned to continue in this manner indefinitely unless histologic evidence of invasion develops

2 Diagnosis of carcinoma of the cervix is more frequently made at an early stage of the disease when cytologic screening is applied

3 In this study the average age of the patients with carcinoma *in situ* was 6 3 years younger than the average age of patients with invasive carcinoma of the cervix

4 Simple suction of the vaginal pool provides adequate specimens for cytologic review and may be done by a nurse or technician Actual exposure of the cervix and scraping of its surface yields no significant increase in number of neoplasms

5 Clinical judgment even when aided by such adjuncts as the Schiller iodine test, is wholly unreliable in estimating the presence of carcinoma *in situ*

6 It is necessary to exclude by careful histologic study actual invasive carcinoma of the cervix, endocervix or endometrium before definitive treatment is decided upon

7 Cytologic screening is an excellent method of selecting from a general population the vast majority of individuals who either have early malignancy of the cervix or deserve further study It is not felt that cytology alone is sufficient evidence for the institution of definitive therapy

REFERENCES

1 Meigs J V Tumors of the female pelvic organs New York The MacMillan Company 1934

2 Reagan J W and Hicks D J A study of *in situ* and squamous cell cancer of the uterine cervix Cancer Phila *6* 1200 1214 1953

3 Reagan J W Carcinoma *in situ* Year book of pathology and clinical pathology 1954 55 series Chicago Year Book Publishers Inc 1955 pp 209 221

4 Fennell Jr R H Carcinoma *in situ* of the cervix with early invasive changes Cancer Phila *8* 302 309 1955

5 Foraker A G Comparison of histochemical properties of normal and neoplastic squamous epithelium Am J Clin Path *31* 581 1955

6 Friedell C H and McKay D G Adenocarcinoma *in situ* of the endocervix Cancer Phila 6 887 897 1953

7 Weston J T and Guin C H Epithelial atypias with chemotherapy in 100 acute childhood leukemias Cancer Phila *8* 179 186 1955

ELECTROLYTE METABOLIC BALANCE STUDIES OF THE POSTPARTUM PATIENT*

JOHN C BUCKINGHAM AND ALLAN C BARNES

The present report is concerned with balance studies on sodium potassium and nitrogen measured during the immediate puerperium Along with the recent work by Dieckmann concerned with the compartmentilization of body fluids and electrolytes [1] there are two earlier reports on the balance of the postpartum patient by Hummel *et al* [2] and Taylor and associates [5] Previous knowledge as to the electrolyte status of the puerperal patient has derived entirely from output studies measuring urinary excretion without regard to dietary intake during the period of study The importance of the puerperium is obvious since any major alterations in balance could presumably reflect the metabolic status during the latter portion of pregnancy

METHOD

Most of the patients in the present investigation were admitted to the metabolic ward within 1 hour after delivery [3] As a result they had been fasting for approximately 20 hours prior to the start of the study Since most of the deliveries were done under conduction anesthesia a full meal was presented to these patients after their arrival in the ward and they were able to assume a full metabolic load at once All of the patients were ambulated within 6 to 12 hours after being admitted to the metabolic

*From the Department of Obstetrics and Gynecology Western Reserve University Medical School Cleveland Ohio

ward and the degree of activity of the group was maintained at about the same level[4] Dietary intake of all sources of sodium, potassium and nitrogen were recorded measurements, in addition to urinary excretion studies included washing out the perineal pads and measuring the electrolyte content of the wash water and breast milk losses in those mothers who were nursing their offspring

A total of 16 women are included in the present study Of these, 27 were normal primiparous patients and 10 were normal multiparous patients Five patients were pre eclamptic ranging from mild to moderate and 1 patients were studied after puerperal hysterectomy

RESULTS

In considering the results the non toxemic patients regardless of gravidity can be grouped together These 37 patients showed a wide variation in their immediate puerperal response The nitrogen balance findings Hummel, *et al* observed with their single patient could not be sustained in this larger group The same trend of electrolyte balance results reported by Taylor and associates in their 3 normal patients, however, was observed in our study[5,6] Individual day by day variations were surprisingly wide and could not be correlated with the parity duration of labor, nor with the estimated blood loss at the time of delivery Patterns of behavior which were more or less typical for the group could be delineated but average or mean values were relatively useless in the face of the wide variations in individual response observed The postpartum diuresis tended to appear earlier than anticipated being more marked on the first and second postpartum days The mothers daily weight similarly showed its maximal drop during the first 2 days and then tended to level off

Sodium tended to be excreted in excess of intake in the first 24 hours after delivery with less of a negative balance the next day and then moved towards minor degrees of increased elimination over intake With respect to the sodium balance, the first 1 to 5 days in the puerperium showed an increased elimination over intake of 0 1 equivalent as an average for this group of patients Both the nitrogen and the potassium would not infrequently show negative balances in the period of the first 21 hours after delivery although in many instances these 2 ions would be retained to a greater extent than they were eliminated even at this time Subsequently they would approach a positive balance with increased retention over elimination The total balance of nitrogen and potassium for the initial 5 puerperal days would in almost all instances, show a greater intake than there had been elimination

In the group that had experienced pre eclampsia prior to delivery the pattern of changes was quite similar although somewhat more marked in the first 48 hours The diuresis would be greater at this time as would be the weight loss The negative sodium balance would in most instances be more marked although the total change for the first puerperal week would show an increased elimination over intake which was not too different in the 5 pre eclamptic patients from that seen in the non toxemic control group Nitrogen and potassium again showed a positive balance—the intake exceeding the output for most of the early puerperal days

In the past it has been suggested that the potassium and nitrogen elimi

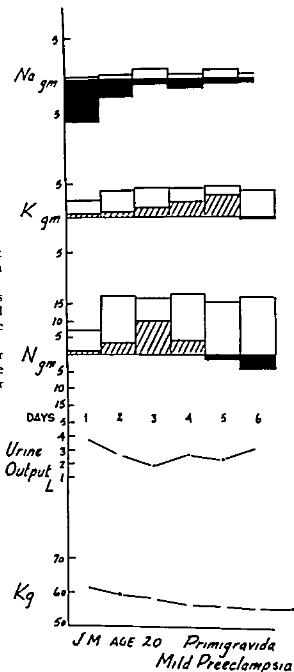


Fig 1 Balance studies for the first 6 puerperal days of a patient with mild pre-eclampsia The dark shading beneath the base line indicates the excess of the electrolyte spilled per day the cross hatchings above the line indicate the amount retained with an excess of intake over output The pattern for each of the ions is the reverse of that seen after gynecologic surgery

nation of the immediate puerperium was predominantly due to the involution of the uterus with the associated protein breakdown Accordingly a group of 4 patients who had either puerperal hysterectomy in the first 24 hours postpartum or Cesarean hysterectomy were admitted to the balance ward for additional study After an initial period of readjustment these patients showed findings that were surprisingly like the findings in the control group There was in most instances a rather sharp elimination of nitrogen and potassium in the first post operative days but subsequently these patients would assume a balance pattern tending toward elimination of sodium and an increased retention of both nitrogen and potassium The total electrolyte balance for the period of time that the patient was

in the hospital would actually be close to balance zero this was undoubtedly influenced by the fact that these patients remained in the hospital for a longer period of time than did the patients delivered vaginally

COMMENT

The present study represents work in progress and the observations made must be largely tentative

(a) The wide variability in balance study response of the puerperal patient tends to preclude sweeping conclusions about the metabolic readjustments of the puerperium in all pregnant patients

(b) In general the normal control patient shows a tendency towards early diuresis with an increased elimination of salt and a retention of both potassium and nitrogen

(c) The patients with pre eclampsia showed no results that were proportionate to the severity of this *complication* *In general*, their electrolyte balance pattern was exceedingly similar although there was a greater tendency to eliminate sodium and fluid in the first 24 hours than there was in the non toxemic patient The nitrogen and potassium metabolism was positive in most cases

In the group of patients with puerperal hysterectomy the initial readjustment not infrequently was slightly more prolonged but at the end of 24 to 48 hours in most instances they were similarly excreting an excess of sodium while retaining both nitrogen and potassium

In conclusion these changes would seem to reflect the abrupt withdrawal of a high level of steroids all of which produce a retention of sodium with an increased elimination of potassium The amount of blood lost at delivery the duration of the labor, and even the inclusion of a postpartum hysterectomy seemed to be overshadowed by the impact of the rapid decline in steroids which is associated with the delivery process

REFERENCES

1 Dieckmann W J Electrolyte balances and the body fluids in pre eclampsia Am J Obst (in press)

2 Hummel F C Sternberger H R Hunscher H A and Macy I G Metabolism of women during reproductive cycle VII Utilization of inorganic elements (A continuous case study of a multipara) J of Nutrit *11* 235 1936

3 Barnes A C Production of ACTH in the patient undergoing gynecologic surgery or receiving pelvic irradiation Am J Obst *65* 758 April 1953

4 Hegsted D M False estimates of adult requirements Nutrit Rev *10* 257 1952

5 Taylor H C Jr Warner R C and Welsh C A The relationship of the estrogens and other placental hormones to sodium and potassium balance at the end of pregnancy and in the puerperium Am J Obst *38* 748 1939

6 Taylor H C Jr Warner R C and Welsh C A The relationship of the estrogens and progesterone to the edema of normal and toxemic pregnancy Am J Obst *45* 547 1943

HORMONAL METABOLISM IN PREGNANCY*

E. JURGEN PLOTZ

The availability of isotopically labelled compounds has provided an ideal tool for the study of the biosynthesis and metabolism of steroid hormones. In his classic experiment Konrad Bloch[1] administered cholesterol labelled with deuterium to a woman in the 8th month of pregnancy. Pregnane-3(α) 20(α)-diol isolated from the urine contained significant concentrations of deuterium, suggesting the probable conversion of cholesterol to progesterone, the principal precursor of pregnanediol. The close cooperation of 3 research groups at the Chicago Lying In Hospital (M. Edward Davis), the Argonne Cancer Research Hospital (C. V. LeRoy, H. Werbin), and the Los Alamos Scientific Laboratory (R. G. Gould) has contributed to the understanding of the biogenesis and metabolism of steroid hormones during human pregnancy by using tagged compounds and thus has led to the results that are discussed in this paper.

METHOD

The experimental procedure, biochemical methods of isolation and identification of compounds, and methods of radioassay used in this investigation are described in greater detail elsewhere.[3,4] Briefly, a single injection of sodium acetate labelled with C^{14} at the carboxyl group (1-C^{14}-acetate) was administered intravenously to pregnant patients who were scheduled for therapeutic termination of pregnancy for various reasons. A dose of 200 μc 1-C^{14}-acetate was used in all patients. Pregnancy was terminated about 2½ to 3½ hours after injection. In some patients cholesterol labelled with tritium (T-cholesterol) in doses of 5.5 to 10.0 μc per day were administered orally for a period of 8 to 14 days before surgery. Cholesterol isolated from blood and tissue samples and urinary steroids excreted into the urine were radioassayed for tritium and C^{14} using a liquid scintillation counter.[5] In other patients progesterone labelled with C^{14} at carbon position 4 (C^{14}-progesterone) was administered intramuscularly. Steroids carrying the C^{14} label were isolated from the urine of the patients. The concentration of C^{14} and tritium in a sample (specific activity) was expressed in μc per mMole. The tracer doses used were approved by the Radioisotope Committee of the University of Chicago Clinics.

RESULTS AND DISCUSSION

Synthesis of Cholesterol in Maternal and Fetal Organs. Cholesterol is regarded as an important precursor of steroid hormones. Therefore it is important to learn as much as possible about the rate of synthesis in various endocrine organs of the mother and the fetus. Figures 1 and 2 show the specific activity of C^{14}-cholesterol derived from 1-C^{14}-acetate in tissues from 2 representative cases. The highest specific activity for free C^{14}-cholesterol was found in the corpus luteum of pregnancy, indicating a very fast rate of incorporation of 1-C^{14}-acetate in cholesterol in this gland. The fetal pla-

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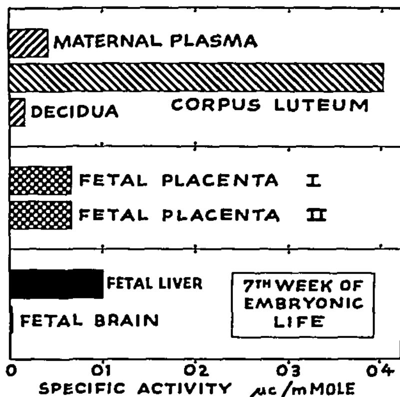


Fig 1 Free C^{14} cholesterol in maternal and fetal tissues after a single injection of 200 μc. 1 C^{14} acetate

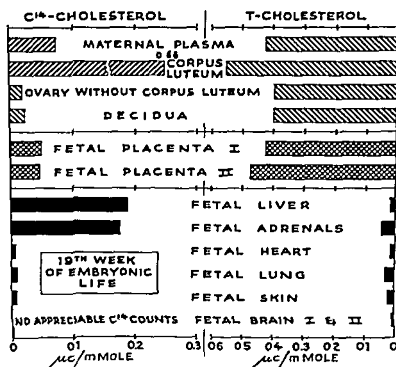


Fig 2 Double label experiment Free C^{14} cholesterol and free T cholesterol in maternal and fetal tissues

centa is able to synthesize cholesterol from acetate early in pregnancy (Fig 1) since the specific activity of placental free C^{14} cholesterol is higher than that of plasma C^{14}-cholesterol However the maternal part of the placenta the decidua appears not to synthesize cholesterol from acetate The fetal liver and the fetal adrenals utilize acetate administered to the mother for the synthesis of cholesterol while cholesterol formation from acetate in other

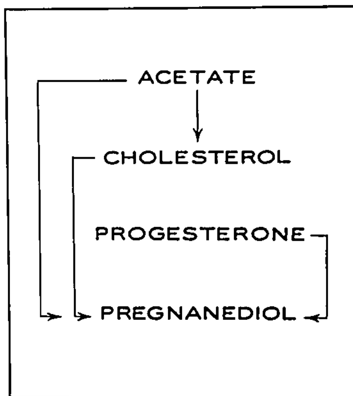


Fig 3 Precursor product re actions concerning synthesis and metabolism of progeste rone in human pregnancy

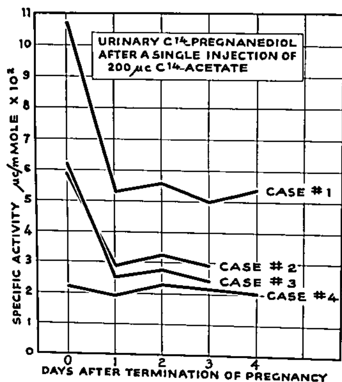


Fig 4 Urinary C pregnane 3(α) 20(α) diol after a single injection of 200 µc 1 C acetate 2½ to 3½ hours before termination of pregnancy

fetal organs (skin brain heart lungs and aorta) was not demonstrated As shown in Figure 2 the fetal placenta absorbs maternal cholesterol to a large extent However the specific activity of free T cholesterol found in other fetal organs indicates that a relatively small percentage of cholesterol present was derived from the mother

Acetate and Cholesterol as Precursors of Progesterone When T cholesterol

was administered orally to pregnant patients for a period of 8 to 11 days pregnane-3(α),20(α)-diol labelled with tritium was isolated from the urine, confirming the results of Bloch[1] who used the stable isotope deuterium to label the cholesterol administered. When 1-C^{14} acetate was injected in the same patients appreciable radioactivity from C^{14} was found in the urinary pregnane-3(α),20(α)-diol samples. Pregnane-3(α),20(α)-diol is regarded as the principal metabolite of progesterone. Therefore C^{14} progesterone was administered intramuscularly to a patient about 2 days before therapeutic interruption of pregnancy was performed. Pregnane-3(α),20(α)-diol isolated from the patient's urine showed an appreciable concentration of C^{14}, which demonstrated the conversion of progesterone to pregnanediol. Radioactivity from C^{14} was also obtained in a fraction of the urinary α-ketonic steroids that very likely represents another metabolite of progesterone: pregnane-3(α)-diol-20-one. Figure 3 illustrates the findings concerning synthesis and metabolism of progesterone.

Figure 1 shows the specific activities of C^{14} pregnane-3(α),20(α)-diol excreted into the urine of pregnant women following the intravenous injection of 1-C^{14} acetate to various patients shortly before termination of pregnancy. The interval between injection and termination of gestation varied between 2½ and 3½ hours. Therapeutic interruptions of pregnancy were carried out in case Nos. 1, 2 and 3 during the 7th to 12th week of embryonic life. In case No. 4 an anencephalic fetus was spontaneously delivered during the 31th week of embryonic life. The acetate was administered after labor had begun.

The specific activity of C^{14} pregnane-3(α),20(α)-diol excreted on the operation day by way of the urine of patients Nos. 1, 2 and 3 was higher than that of C^{14} pregnanediol excreted by patient No. 4 although the same dose of 200 μc 1-C^{14} acetate had been given to all 4 women and the interval between injection and termination of pregnancy was almost the same. This finding indicates that the rate of conversion of C^{14} acetate to C^{14} pregnanediol was slower during spontaneous labor as compared with that during the 7th to 12th week of pregnancy.

The specific activity of C^{14} pregnane-3(α),20(α)-diol excreted in the urine during the postoperative days remained constant in case No. 4 which indicated that no further synthesis of pregnanediol from acetate and other precursors took place after spontaneous termination of pregnancy. However, the specific activity of C^{14} pregnanediol decreased significantly to lower values on the first postoperative day in case Nos. 1, 2, 3 and remained constant during the following days. This decrease in the specific activity suggests that pregnanediol was still synthesized from nonradioactive precursors *thus producing a dilution of the concentration of* C^{14} pregnanediol molecules in the urinary pregnanediol sample.

It is rather tempting to conclude from the results of these experiments that the rate of synthesis of progesterone, the principal precursor of pregnanediol, was relatively slow during spontaneous labor although the absolute amount of urinary pregnanediol was still large at the time of delivery. This conclusion would be correct provided (1) that in all patients no hormonal precursors other than progesterone were converted to pregnanediol to an appreciable extent and (2) that progesterone formed from acetate was metabolized to pregnanediol at the same percentage rate in all instances.

Our findings suggest strongly that pregnanediol was synthesized from nonradioactive precursors (progesterone?) on the first postoperative day in case Nos 1 2 and 3 but not in No 1 The source of these precursors in case No 2 may have been part of the corpus luteum that had been removed incompletely In case No 3 an hysterotomy and an extirpation of the ovary containing the corpus luteum was performed indicating the possibility that the precursors were synthesized in syncitial tissue commonly present in the myometrium after the separation of the placenta In case No 1 however, the uterus and the ovary containing the corpus luteum were extirpated Therefore another source of precursors of pregnanediol synthesized on the first postoperative day must be taken into account most likely the maternal adrenal cortex

SUMMARY

Cholesterol is synthesized by the corpus luteum and the fetal part of the placenta early in human pregnancy There is no evidence of cholesterol synthesis from acetate in the maternal part of the placenta the decidua The fetal liver and the fetal adrenals utilize acetate for the formation of choles terol Fetal skin and brain do not produce amounts detectable with this method It appears that a relatively small percentage of fetal cholesterol is derived from maternal cholesterol in early pregnancy indicating that mater nal cholesterol passes the placenta at this stage of pregnancy

Acetate cholesterol and progesterone labelled with C^{14} or tritium serve as precursors of isotopically labelled pregnane 3(α) 20(α) diol excreted into the urine of pregnant patients The rate of synthesis of pregnanediol appeared to be rather slow during labor as compared with the rate of pregnanediol formation in normal pregnancy during the 7th to 12th week of gestation This finding may permit the tentative conclusion that progesterone the principal precursor of pregnanediol is synthesized at a slower rate during labor than in early pregnancy

REFERENCES

1 Bloch K The biological conversion of cholesterol to pregnanediol J Biol Chem *157* 661 666 1945

2 LeRoy G V Gould R G Bergenstal D M Werbin H Davis M E Plotz E J and Kabara J J The use of tritium and radiocarbon labelled precursors to study the metabolism of steroid hormones in man I Extrahepatic synthesis of cholesterol in man In preparation

3 Gould R G LeRoy G V Okita G T Kabara J J Keegan P and Bergenstal D M The use of C^{14} labelled acetate to study cholesterol metabolism in man J Laborat Clin M *46* 372 390 1955

4 LeRoy G V Werbin H Davis M F Plotz L J and Bergenstal D M The utiliza tion of exogenous cholesterol for the formation of adrenal cortical hormones in man Annual Meeting of the Central Society for Clinical Research November 1954 Chicago

5 Hiebert R D and Watts R J Fast coincidence circuit for H^3 and C^{14} measurements Nucleonics *11* 38 41 1953

Neurological Surgery

INTRODUCTION

C. Hunter Shelden

The neurosurgical portion of the Forum program has become increasingly popular during the past few years.

This year's program again deals with surgical problems of the nervous system which are of interest to all surgeons. Hypotension and artificial hibernation are discussed not only from the general standpoint but also with regard to their specific application to intracranial operations. Hypothermia probably will prove as valuable for intracranial vascular procedures as it now is for cardiac surgery.

One session of the Forum is confined to reports of investigative procedures in the field of neurosurgery. This serves as a valuable source for information concerning recent investigative work. It serves as a weather vane indicating the newer trends in neurosurgery. The Forum thereby offers opportunity for neurosurgical investigators to present briefly valuable material not elsewhere available and at the same time affords an equally valuable means of exchange of information regarding problems of common interest.

THE CHOROID PLEXUS — A POTENTIAL ORGAN OF EXCRETION*

O. HUGH FULCHER

Many methods for increasing needed body fluids have been devised during the past 75 years. Occasionally there have been patients who have urgently required elimination of excessive body fluids when their kidneys have failed temporarily to function adequately. Under these conditions the accessory methods of stimulating excretion of fluids by respiration, by sweating and by catharsis have rarely proved effective. The desperate demand for a practical solution to the problem of combating generalized edema of a patient in a state of anuria was inflicted upon me by an intimate friend during the year 1950. This young man had developed anuria insidiously and had become waterlogged shortly after his admission to the hospital. The medical consultants attempted to stimulate excretion of the body fluids by the means mentioned above. Also they attempted to produce localized edema in the extremities by use of tourniquets. The artificial kidney was employed with no success. The patient died. Autopsy revealed lower nephron necrosis, generalized edema and much fluid in the body cavities. Furthermore, there was evidence that the renal tubules were regenerating and could have functioned again had the patient lived a few days longer. This tragedy stimulated me to attempt to discover other methods for excretion of body fluids.

I thought of the cerebrospinal fluid as representing a closed lake within the interior of the body. This lake, under normal conditions, is supplied by the arterial system in the form of the choroid plexus and drained into the venous system through the arachnoid villi, the walls of the subarachnoid veins perhaps, and by way of the perineural lymphatics.

The choroid plexus has an anatomical structure resembling the renal glomeruli and the filtrate of each is derived from the same blood serum. The filtrate of the choroid plexus might possibly be identical to that of the glomerulus if the pressure necessary for venous absorption could be eliminated.

If the cerebrospinal fluid could be drained to the outside, then the choroid plexus would be an organ of excretion. The cerebrospinal fluid could then become a modified urine to supplement renal function by getting rid of some body fluids with its contents of the products of metabolism.[1]

METHOD

The patients with communicating hydrocephalus who had experienced the Matson type of arachnoid ureterostomy to drain the excessive fluid into the urinary bladder had demonstrated that the constant loss of cerebrospinal fluid was compatible with life. These patients had required increased ingestion of sodium chloride because of the excessive loss. They had demonstrated also that the polyethylene tube was well tolerated by the subarachnoid spaces and that it would remain patent for years. Therefore the problem consisted of temporarily simulating the permanent environment of the polyethylene tube used in the Matson operation.

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Spinal punctures performed on several of the patients who had been operated upon for communicating hydrocephalus revealed the pressure of the cerebrospinal fluid to be 50 to 90 mm of water This positive pressure probably prevented the collapse of the subarachnoid spaces and the plugging of the openings in the polyethylene tube by the floating nerves of the cauda equina

I am now using the following method of drainage of the cerebrospinal fluid under controlled pressure which was gradually evolved by experience and disappointments (Fig 1)

A #3 8F or 1 7F polyethylene tube 15 inches long is used Several perforations are made with a paper punch apparatus within 2 cm of the end This 2 cm portion of the tube is then bent at right angles by heating with hot water The tube is then sterilized by use of aqueous zephrin and subsequently thoroughly rinsed in normal saline solution A lumbar puncture is performed between the third and fourth lumbar spinous processes with a #16 gauge Tuohy spinal puncture needle (a #11 gauge needle is required for the large tube) The tube properly marked is threaded through the needle so that the bent portion extends toward the *cul de sac* of the arachnoid The needle is withdrawn and the tube is anchored to the skin with a Tuohy Borst adaptor to which has been welded a steel flange to serve as the base Over this is placed a transparent plastic hemisphere to prevent kinking of the polyethylene tube and to protect the modified adaptor should the patient turn on his back The transparency of the hemisphere permits inspection The polyethylene tube emerges through a little notch in the

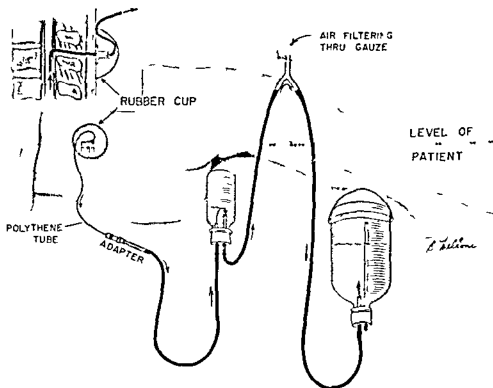


Fig 1

border of the plastic hemisphere and the other end is connected to the ordinary rubber tubing by a Tuohy Borst adaptor This leads to a closed inverted bottle which is located below the level of the patient Another rubber tube connects with an inverted Y glass tube which is kept above the level of the spinal puncture A short rubber tube is connected to the other arm of the inverted Y glass tube which leads to an inverted intravenous bottle kept at a lower level The vertical arm of the inverted Y tube is protected by sterile gauze and it admits air to prevent siphonage The inverted intravenous bottle can be changed without contaminating the fluid or disrupting the system If the intravenous bottle should become over filled the fluid will spill on the floor without exerting any back pressure

The spinal subarachnoid pressure is controlled by the height of the inverted Y glass tube The same apparatus can be used for introducing fluid into the subarachnoid space by altering the height of the inverted intravenous bottle

A specimen of blood serum and of spinal fluid of each patient was used at the beginning of the study and at 24 hour intervals thereafter These clinical studies were performed by Dr Alvin Parrish and we shall publish the results in a separate report

RESULTS

Continuous subarachnoid drainage under controlled pressure has been used on 9 patients for periods varying from 24 hours to 9 days The methods used with the first patients were inadequate There has persisted until recently the problem of preventing the kinking of the polyethylene tube while treating unconscious and uncooperative patients

Two of these patients who had normal kidney functions were used as controls One patient had advanced cardiac failure 1 patient had severe arteriosclerosis and psychosis complicated by acute bromide poisoning and 1 patient 29 years of age whose blood pressure was 240/160 was suffering with the terminal stage of essential hypertension She presented papill edema and severe retinal hemorrhages The other 4 patients were suffering with severe arteriosclerosis and had high nonprotein contents of the blood sera I have not yet encountered the ideal patient suffering with lower nephron necrosis

The amount of cerebrospinal fluid excreted depended somewhat upon the subarachnoid pressure maintained the amount of fluid intake of the patient and the state of renal function

Continuous subarachoid drainage appeared to stimulate excretion of poorly functioning kidneys by some means quite unexplainable The control patients excreted cerebrospinal fluid at approximately one third that of urine The young patient with hypertension excreted 800 cc of cerebrospinal fluid as compared to 350 cc of urine during 24 hours This patient had severe damage of the retinal vessels and presumably of the choroid plexus All other patients excreted from 250 to 800 cc of cerebrospinal fluid during a 24 hour period

Dr Parrish has found that the urea nitrogen contents of the serum and of the spinal fluid were always identical the sodium and chloride contents were a little higher in the spinal fluid the potassium and creatin contents were less

The senile patient suffering with a serum content of sodium bromide of 700 mg per cent excreted this bromide readily in the cerebrospinal fluid and quickly recovered

One patient who was slightly jaundiced excreted bile in the cerebrospinal fluid at the same concentration as it occurred in the serum

Three of the patients were moribund at the time of the study The autopsies revealed marked dilatation of the cortical veins The young patient with severe hypertension revealed slight necrosis of the cerebellar tonsils

DISCUSSION

The excretion of the cerebrospinal fluid should always occur under slightly positive pressure to avoid plugging of the polyethylene tube in the subarachnoid space by the floating nerves to avoid collapse of the subarachnoid spaces, and to prevent herniation through the foramen magnum or incisura

The positive pressure should not exceed 30 mm of water lest venous absorption of the cerebrospinal fluid be promoted

This apparatus may be used to study the secretion of cerebrospinal fluid under various conditions and to increase subarachnoid pressure during intracranial hypotension should this be desirable

Continuous drainage of cerebrospinal fluid may be used on patients suffering with communicating hydrocephalus to determine beforehand if permanent arachnoid ureterectomy will constitute successful surgical therapy

Cushing Wallace and Brody have demonstrated that the choroid plexus is able up to a certain threshold value to retain from the cerebrospinal fluid agents noxious to the nerve cells the chemical compositions of which would not prevent their passing a semipermeable membrane Some of these noxious agents are iodides salicylates nitrates bile pigments and ferments The jaundiced patient did excrete bile pigments readily in the cerebrospinal fluid This observation could indicate that the threshold of the blood brain barrier is diminished or possibly eliminated if the cerebrospinal fluid is drained under atmospheric pressure

The drainage of cerebrospinal fluid under controlled pressure converts it to a modified urine and thus the choroid plexus becomes an organ of excretion It is evident however that the choroid plexus can never replace the kidney The sodium and chlorides are excreted in abundance Adequate fluid can be excreted to prevent edema but since the approximate contents of urea, potassium and creatine of the cerebrospinal fluid are respectively identical to those of the serum there will be a gradual accumulation of the waste products of metabolism Therefore the use of the spinal fluid as a modified urine may relieve to some extent overburdened kidneys or may permit a patient to survive lower nephron necrosis until the tubules can regenerate yet it can never carry the whole load This method of cerebrospinal fluid drainage may therefore be beneficial to patients who are suffering with temporary renal failure but it will be of little value to those who are suffering with chronic renal failure

The autopsies revealed that cerebral veins had become dilated and thus it is conceivable that these structures could contribute to the formation of subarachnoid fluid thus reversing their usual role

This method to relieve overburdened kidneys does not alter the circula

tion but permits the blood to remain in its normal channels throwing no excess strain on the heart and permitting the filtration or dialysis of the serum to occur under normal conditions with nature s equipment It can be continued almost indefinitely

The method described above can be employed in the home or hospital by anyone who can do a spinal puncture The total cost of the materials required will be only a few dollars The apparatus requires about the same supervision as does an indwelling catheter

SUMMARY

A method of continuous drainage of the subarachnoid spaces under con trolled pressure has been described This method permits the cerebrospinal fluid to become a modified urine and the choroid plexus to become an organ of excretion It can be used anywhere by anyone who can do a spinal puncture it requires little supervision It does lower the threshold of the blood brain barrier The materials required are a #16 gauge Tuohy spinal puncture needle a #3 8F polyethylene tube a modified Tuohy Borst adaptor a transparent plastic hemisphere a Tuohy Borst adaptor ordinary rubber tubing a closed bottle with stopper equipped with 2 glass tubes of equal length a Y tube and an empty intravenous bottle

CONCLUSIONS

The subarachnoid spaces can be drained under controlled pressure thus converting the cerebrospinal fluid into a modified urine and the choroid plexus into an organ of excretion Adequate fluid sodium and chlorides can be excreted in this manner The cerebrospinal fluid has about the same content of urea creatine and potassium respectively as does the serum The employment of the choroid plexus as an accessory organ of excretion is beneficial to patients suffering with bromide poisoning or temporary renal failure

REFERENCES

1 Voetmann Edel On the structures and surface area of the human choroid plexuses A quantitative anatomical study Acta anat Supp L Basel *8* 10 11 1949

2 Matson D D A new operation for the treatment of communicating hydrocephalus report of a case secondary to generalized meningitis J Neurosurg *6* 238 247 1949

3 Cushing H Studies in Intracranial physiology and surgery Oxford Med Publ London Humphrey Milford 1926 p 137

4 Wallace G B and Brodie B B The distribution of iodide thiocyanate bromide and chloride in the central nervous system and spinal fluid J Pharm Exp Ther *65* 220 1939

PHYSIOLOGICAL AND THERAPEUTIC EFFECTS OF BILATERAL INTERMEDIATE MIDBRAIN CRUSOTOMY FOR ATHETO DYSTONIA (17 Cases)*

RUSSELL MEYERS

This paper reports the early clinical neurophysiologic and psychosociologic therapeutic results noted in a series of 17 cases of severe hyperkinetic cerebral palsy subjected within the past 3 years to bilateral two-staged surgical interruption of the intermediate three fifths of the crus cerebri — an operation for which the term intermediate crusotomy is herein proposed

Bakwin and Bakwin (1951) estimate that approximately half of all patients suffering from cerebral palsy exhibit some degree of hyperkinesia and disorder of striated muscle tonus Among these choreatic athetotic and dystonic movements are by far the most common

The patients dealt with in this series exhibited the most severe degrees of such disturbances Axial and appendicular muscles were involved to such an extent as to disrupt severely postural set and to preclude all purposive and adaptive movements Hence in a practical sense all patients were quadriplegic i e were wholly dependent upon others for eating dressing and hygienic measures With advance in years and corresponding increase in weight and motor power, they became increasingly more difficult problems in management Their unpredictable and uncontrolled movements led at times to injuries ranging in severity from sheet burns to dislocations of limbs

In all instances speech was severely dysarthric and the patients had been considered largely beyond education and habilitation Their abnormal movements similarly interfered with the pursuit of pleasurable recreational and social experiences Finally, psychosociologic consequences were increasingly felt by the patients families especially as various physical orthopedic surgical pharmacologic and psychoeducational therapeutic endeavors repeatedly proved disappointing

A survey of the surgical literature bearing upon bilateral choreatic athetotic and dystonic hyperkinesias was not encouraging Tenotomies myotomies arthrodeses neurectomies sympathectomies and rhizotomies variously employed in the past were obviously limited in application to circumscribed bodily segments Cortical ablations of the motor and premotor cortex introduced by Horsley in 1890 and 1909 and subsequently developed by Bucy (1932) and others involved a substitution of one neurologic deficit by another serious but more acceptable disorder In addition the cortical procedures invited postoperative epilepsy Bucy asserted cortical ablation to be applicable only to unilateral cases Putnam (1933) succeeded in dampening but not abolishing choreoathetotic movements in over two-thirds of his cases subjected to high cervical anterolateral cordotomy A subsequent follow up of his series indicated that in some cases early gains were lost in whole or in part Further cordotomies while carrying no potential for relieving hyperkinesias of the face jaw tongue and eyes entail acceptance of extensive analgesia and thermanesthesia as well as the described motor deficits The operation of hemispherectomy as employed by Krynauw

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(1950) for infantile hemiplegia with attendant convulsions and behavior disorders seemed clearly inapplicable to bilateral cases of the sort with which we were dealing. Again, ligation of the anterior choroidal artery as employed by Cooper (1953) has since proved unsuccessful in cases of choreoathetosis and dystonia.

The present writer's previous attempts to deal with the choreoathetotic and dystonic hyperkinesias included (1) subpial linear section through the crown and along the length of the precentral gyrus sectioning the cortex and underlying U fibers to a depth of approximately 2.5 cm; (2) subpial linear section as in (1) above followed by undercutting the cortex considered to correspond to Brodmann's Area 6; (3) pallidofugal section (ansotomy), and (4) extirpation of the head of the caudate nucleus and curvilinear section of the anterior limb of the internal capsule as far posteriorly as the capsular genu. These experiences have been previously reported upon (Meyers 1942, 1951). While certain of them appeared very useful for parkinsonism and hemiballism, they had proved largely inept for the control of choreoathetotic and dystonic movements.

Walker (1949) had previously reported amelioration of a case of hemiballismus in which he sectioned the lateral half to two-thirds of the crus cerebri of one side. The possibility of utilizing this for cases of athetodystonia was considered. However, lateral pedunculotomy did not appear to warrant much optimism in the latter disorders in view of its failure to improve a dystonic patient reported upon by the present writer in 1951.

This overall review of previous failures prompted speculations upon a more promising surgical procedure, the "realistic ideals" of which were to include the following:

1. Abolish or at least markedly reduce hyperkinesia and dystonia so as to minimize self injury of patients.
2. Relax contractures and postural deformities.
3. Make possible a more facile management of the patients by parents and attendants.
4. Make it possible for the patient to sit quietly so that entertainment, educational and occupational programs might be implemented in his behalf and social tensions eased for those in his familial environment; and
5. Achieve some measure of motor control such that intended acts, however crude, might be carried to completion.

In addition (and again ideally) certain consequences of operation were clearly to be avoided. These were:

1. Further impairment of intellectual capacity such as might follow surgical damage to the neocortex.
2. Loss of such functions as might already prevail, e.g., speech, deglutition, sphincter control, ability to initiate acts, etc.
3. Post-operative epileptiform seizures.
4. Autonomic (vegetative) instabilities, e.g., of temperature control, blood pressure, fluid and electrolyte balance, etc.

The notion of interrupting the pyramidal tracts bilaterally in the intermediate three-fifths of the midbrain crura was now entertained. Arguments against such a procedure, especially anticipation of complete volitional paralysis, did not, in view of previous considerations (Meyers 1953), appear to the writer substantial. Accordingly the procedure was carried out on 8

males and 9 females ranging in age from 3 to 25 years Preoperative work ups included electroencephalography motion picture studies and sound track recordings of speech and psychometry The second stage (contra lateral) crusotomy was performed in from 3 to 6 months following the initial unilateral crusotomy

The results are as follows

Markedly improved	13	Compared with ideal criteria set forth above
Moderately improved	3	
Slightly improved	1	
Operative mortality	2	

The slightly improved case was one in which in an effort to ascertain effectiveness of a minimal procedure only two fifths of the crura were sectioned

Each of the patients who died did so following the second stage of operation having shown a measure of clinical improvement during the interval between the first and second stages One of these a female aged 3 years died a week after operation as a result of an acute massive gastro intestinal hemorrhage The cause of death in the other patient a female aged 22 years was not determined

It is noteworthy that in all cases showing abolition of abnormal move ments on the side contralateral to the initial crusotomy an appreciable dampening of abnormal movements was evident on the *ipsilateral* side even before the second stage operation was undertaken Urinary incontinence was a regular sequel following the second stage of operation but was recovered from in all cases before the sixth week

Commonly a flaccid areflexic contralateral limb paralysis persisted for from 1 to 3 weeks following operation As time passed muscle tone and paretic voluntary movements gradually returned to face eyes head neck tongue scapulo humeral and pelvico crural musculature These movements while crude are clearly under the patient s command They prompt serious challenge of the conventional statements which make voluntary move ment the private business of the pyramidal system

The surviving patients are now uniformly easier to manage and can sit for entertainments educational efforts and social intercourse previously closed to them One patient has learned to read since operation another can walk with support and 3 have shown objective improvement in their speech Early operation is indicated in order to provide early opportunities for educational and social experience

CLINICAL RESULTS OF RADIOYTTRIUM HYPOPHYSECTOMY*

Eric T. Yuhl, Paul V. Harper, Theodore B. Rasmussen and
Delbert M. Bergenstal

It is well established that many cancers arising in the breast and prostate gland are subject to endocrine influence. Castration, adrenalectomy and the administration of various hormones have produced prolonged and objective improvement in many of these cases. Hypophysectomy has followed logically as an extension of this approach to cancer therapy. Luft and Olivecrona[1] have shown that surgical hypophysectomy can be performed without serious risk and with encouraging results in many cases. We have attempted to carry out this procedure in a series of 9 cases, in all of which the hypophysis was incompletely removed as shown by serial sections taken through the region of the sella turcica at autopsy. Since the interpretation of the results of this clinical experiment depends on the achievement of complete hypophysectomy, a more certain method of accomplishing this result was sought.

Destruction of the gland with injected radioactive colloidal material such as Au^{198} or $CrP^{32}O_4$ was considered, but it was decided, because of proximity of numerous important parasellar structures, that the destructive action of the radiation field could be better controlled by using discrete sources of beta radiation such as those described by Kisieleski, Swihla and Brues.

METHOD

Yttrium 90 was chosen as the most suitable agent because of its strong beta radiation (2.3 MEV), short half life (62 hrs.) and adequate activation cross section for thermal neutrons (1.24 Barns). Compressed pellets of Y_2O_3 powder were heated for an hour at 1650° C, which converted them to solid ceramic material. Small cylinders, 2 to 5 mg. in weight, were formed in this way to fit into a #17 gauge spinal puncture needle. Activation of these pellets in a thermal neutron flux of 1×10^{13} N/cm²/sec. for 24 hours resulted in a specific activity of 0.4 mc./mg. Y_2O_3 or 1 to 2 mc. per pellet. Handling the activated pellets presented no problem since 11 mm. of water offers complete shielding against the beta radiation. Under water a pellet is placed in a curved spinal puncture needle whose proximal half is enclosed in a 2 mm. lead shield. This reduces the radiation dosage at the operator's finger tips to less than 20 milliroentgens per hour, so that the shielded needle may be handled at operation without haste.

While the maximum range of the beta radiation in tissue is about 1 cm., a significant radiation dosage is not reached until one approaches within 4 or 5 mm. of the pellet. The dose gradient surrounding a representative pellet was measured using photographic film in a lucite phantom. Figure 1 shows isodose curves obtained by this method.

Yttrium pellets were implanted in the region of the hypophysis in monkeys. These became surrounded by a more or less spherical region of

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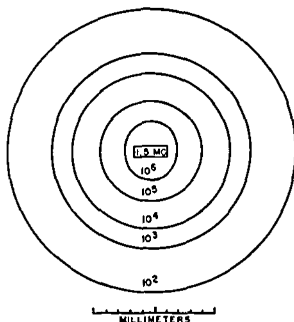


Fig 1 Isodose curves indicating total tissues doses in rep surrounding a 1.5 mc Y^{90} pellet

complete necrosis with very sharply demarcated borders as was expected from the steep gradient of the radiation field The lesions reached their maximum diameter of 1 to 8 mm depending on the activity of the pellet in 7 to 10 days The radiation dosage at the border of the necrotic lesion varied somewhat for different tissues The values measured for the hypophysis and other parasellar structures are shown in Table I

DISCUSSION

On the basis of measurements on typical human pituitary glands it was believed that the whole gland could be destroyed by 4 properly placed 1 mc pellets without risk of radiation damage to the parasellar structures

The operative procedure was carried out through a standard right frontal exposure as in surgical hypophysectomy Following exposure of the chiasm the pituitary stalk was clipped and divided and the pellets were implanted using the shielded spinal puncture needle

At the present time we have carried out this procedure on a total of 21 patients with metastatic cancer

It became evident after the first cases that the 4 pellet plan was inadequate Accurate placement of the pellets was much more difficult than had been anticipated An error of a millimeter or 2 in the placement of the pellets left significant amounts of viable undamaged pituitary tissue In order to overcome this difficulty the number of pellets has been increased gradually from 10 to 12 evenly distributed throughout the gland the total quantity of isotope implanted remaining at 4 to 5 mc In Figure 2 an anteroposterior roentgenogram of the skull shows 7 pellets distributed in

Table 1[5] Radiation Dosage Required to Produce Necrosis

Anterior lobe of hypophysis	110 000 to 190 000 rep
Hypothalamus	60 000 to 120 000 rep
Optic chiasm and tract	60 000 to 140 000 rep
Oculomotor nerve	30 000 to 60 000 rep



Fig 2 Antero posterior view of skull showing distribution of 7 yttrium 90 pellets in pituitary gland



Fig 3 Low power photomicrograph of coronal section through sella turcica showing destruction of pituitary by properly placed yttrium 90 pellets Cephalad sections in this case showed residual gland

the hypophysis Figure 3 shows the degree of destruction obtained with properly placed pellets

Complications attributable to radiation damage have been limited to 4 instances of third or sixth nerve paralysis due to misplacement of pellets and 2 instances of transitory coma due probably to radiation of the hypothalamus by high lying pellets

It is as yet too early to assess the clinical response of the malignant process in most of our cases It may be stated however that patients with incomplete hypophysectomy as shown at autopsy nevertheless show marked endocrine changes as evidenced by hypothyroidism and adrenal insufficiency Diabetes insipidus has been mild and transitory except in those patients with relatively large pituitary remnants

While in none of our patients who has come to autopsy has there been complete destruction of the pituitary the results as judged from the pathological viewpoint have in general been better than in the surgical cases and our recent cases in whom a large number of pellets were used are all still living

CONCLUSIONS

At the present time we believe that the implantation of radioactive yttrium pellets in the hypophysis is as safe a procedure as surgical hypophysectomy is much more rapid once the gland is exposed avoids the danger of hemorrhage from the cavernous sinus and probably offers a better chance for complete hypophysectomy than does the surgical method

REFERENCES

1 Luft R and Olivecrona H Experiences with hypophysectomy in man J Neurosurg *10* 301 16 1953

2 Kisieleski W Sishla G and Brues A M Preparation of radioactive glass beads Science *112* 400 1950

3 Rasmussen T Harper P V and Kennedy T The use of a beta ray point source for destruction of the hypophysis in Surgical Forum 1952 Philadelphia W B Saunders Co 1953 pp 681-686

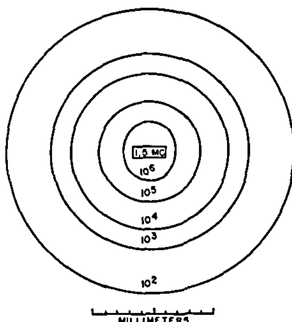


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artery was catheterized and the cisterna magna punctured in such manner as to lose no cerebrospinal fluid Constant pressures from these catheters were measured by utilizing Statham transducers calibrated in mm of mercury and mm of water respectively Tracings were made by heated stylii on a Sanborne recorder At the termination of the recording blood and cisternal cerebrospinal fluid samples were collected and, with the animal still alive the calvarium was removed and the brain rapidly extracted Excess blood was blotted from the specimen which was then divided into 2 parts Samples of gray matter and white matter of approximately 1 gm were separated from each part and the surface pia arachnoid was stripped from the cortex Water content of the gray and white samples was determined by drying in porcelain crucibles at 100° C to a constant weight (18 to 96 hours being adequate) Samples of equal size were weighed and homogenized in a detergent (Sterox) to permit analysis of brain electrolyte content by the Beckman flame spectrophotometer Blood and cerebrospinal fluid samples were analyzed in like manner for electrolyte content

Following the standardization of procedure and the determination of control values the next problem was to induce brain swelling Non lethal non traumatic and non toxic methods were desired The effect of hypotonic solutions administered intravenously upon cerebrospinal fluid pressure to produce an elevation thereof is well known For this reason the solutions utilized were 889 per cent and 445 per cent physiologic salt solution and distilled water They were administered at a constant rate of 60 drops per minute and in a dosage of 40 ml /kg via the anticubital vein at body temperature

RESULTS

Control Observations *Pressure Data* Eleven animals were studied for fluctuation in cisternal pressures in the course of tracings recorded up to 103 minutes duration In the presence of adequate levels of anesthesia a fairly constant cisternal pressure was maintained and fluctuated less than femoral arterial pressure and bore no constant relation to the latter An exception to this occurred when the plane of anesthesia was elevated at which time both cisternal and femoral pressures usually rose and subsequently fell as more pentobarbital was administered A representative tracing of the control animals demonstrating the stable record of cerebrospinal fluid pressure is illustrated in Figure 1

Brain Water and Electrolyte Data A total of 27 animals were analyzed for brain water in the manner described above Serum and cerebrospinal fluid sodium and potassium determinations were obtained in 28 and 31 animals respectively Cerebral gray and white matter determinations for sodium and potassium were obtained in 11 animals These data are presented in Table 1

Experimental Observations The effects of the administration of 889 per cent and 445 per cent physiologic salt solution and distilled water upon the cisternal cerebrospinal fluid pressure are seen in the accompanying reproduction of portions of the original records (Figs 2 3 4) Three animals were administered 889 per cent physiological salt solution 6 animals were given 445 per cent physiological salt solution and 6 animals were given distilled water in the dosage and at the rate noted above The tracings

STUDIES IN CEREBRAL SWELLING I CONTROL STUDIES*

W EUGENE STERN

A project has been outlined to examine the possible relationship between the water content of the brain and the phenomenon of cerebral edema (brain swelling) by which is meant an increase in brain bulk and cerebrospinal fluid pressure in the absence of a local mass lesion or vascular or cerebrospinal fluid obstruction The present paper, the first in a series introduces the project and reports its initial findings

METHOD

Adult healthy mongrel cats of both sexes were utilized in this study In this phase of the study the animals were anesthetized with intravenous injections of pentobarbital sodium (circa 35 mg /kg) The head of the animal was fixed by the holding portion of a stereotaxic apparatus which placed the animal in a prone position with head erect and the level of the cisterna magna above the thorax A femoral artery and the calvarium were exposed and the cisterna magna uncovered without loss of cerebrospinal fluid Sodium heparin (1 mg /kg) was given intravenously the femoral

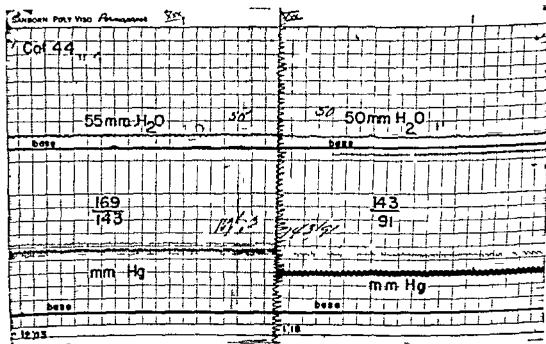


Fig 1 Photograph of original tracing of cisternal pressure and femoral arterial pressure The record is split showing the portion recorded at 12 30 on the left mounted next to right half recorded at 1 18 p m

*From the Department of Surgery Division of Neurological Surgery University of California Medical Center Los Angeles California This project was supported by the Dudley Cates Neurosurgical Research Fund

artery was catheterized and the cisterna magna punctured in such manner as to lose no cerebrospinal fluid Constant pressures from these catheters were measured by utilizing Statham transducers calibrated in mm of mercury and mm of water respectively Tracings were made by heated stylii on a Sanborne recorder At the termination of the recording blood and cisternal cerebrospinal fluid samples were collected and, with the animal still alive the calvarium was removed and the brain rapidly extracted Excess blood was blotted from the specimen which was then divided into 2 parts Samples of gray matter and white matter of approximately 1 gm were separated from each part and the surface pia arachnoid was stripped from the cortex Water content of the gray and white samples was determined by drying in porcelain crucibles at 100° C to a constant weight (18 to 96 hours being adequate) Samples of equal size were weighed and homogenized in a detergent (Sterox) to permit analysis of brain electrolyte content by the Beckman flame spectrophotometer Blood and cerebrospinal fluid samples were analyzed in like manner for electrolyte content

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RESULTS

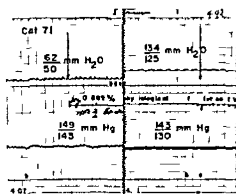
Control Observations *Pressure Data* Eleven animals were studied for fluctuation in cisternal pressures in the course of tracings recorded up to 103 minutes duration In the presence of adequate levels of anesthesia a fairly constant cisternal pressure was maintained and fluctuated less than femoral arterial pressure and bore no constant relation to the latter An exception to this occurred when the plane of anesthesia was elevated at which time both cisternal and femoral pressures usually rose and subsequently fell as more pentobarbital was administered A representative tracing of the control animals demonstrating the stable record of cerebrospinal fluid pressure is illustrated in Figure 1

Brain Water and Electrolyte Data A total of 27 animals were analyzed for brain water in the manner described above Serum and cerebrospinal fluid sodium and potassium determinations were obtained in 28 and 31 animals respectively Cerebral gray and white matter determinations for sodium and potassium were obtained in 11 animals These data are presented in Table 1

Experimental Observations The effects of the administration of 889 per cent and 145 per cent physiologic salt solution and distilled water upon the cisternal cerebrospinal fluid pressure are seen in the accompanying reproduction of portions of the original records (Figs 2 3 4) Three animals were administered 889 per cent physiological salt solution 6 animals were given 445 per cent physiological salt solution and 6 animals were given distilled water in the dosage and at the rate noted above The tracings

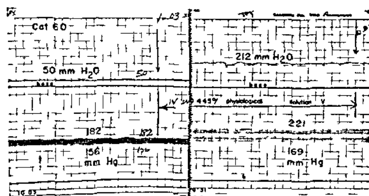
Table 1

DETERMINATION		EXPRESSED AS	NUMBER OF DETERMINATIONS (ANIMALS)	MEAN	STANDARD DEVIATION
Brain water gray		% wet weight	27	78.7	1.1%
Brain water white		% wet weight	27	67.6	1.4%
Serum sodium		mEq/liter	31	157.4	9.0 mEq
Serum potassium		*mEq/liter*	*31*	*3.9*	*0.5 mEq*
C.S.F. sodium		mEq/liter	28	157.8	6.9 mEq
C.S.F. potassium		mEq/liter	29	2.9	0.5 mEq
Brain gray	sodium	mEq/liter	11	61.8	9.3 mEq
	potassium	mEq/liter	11	75.5	9.6 mEq
Brain white	sodium	mEq/liter	11	49.0	11.0 mEq
	potassium	mEq/liter	11	65.5	6.0 mEq



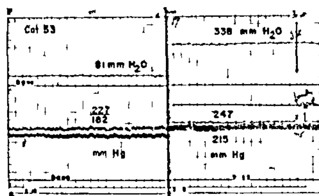
ADMINISTRATION of 0.889% PHYSIOLOGICAL SALT SOLUTION
Split record to demonstrate pre injection C.S.F. pressure and terminal record at completion of drip

Fig. 2. Reproduction of original record cat #71



ADMINISTRATION of 0.445% PHYSIOLOGICAL SALT SOLUTION
Split record to demonstrate pre injection C.S.F. pressure and terminal record at completion of drip

Fig. 3. Reproduction of original record cat #60



ADMINISTRATION of DISTILLED WATER
Split record to demonstrate pre injection
C S F pressure and terminal record at
completion of drip

Fig 4 Reproduction of original record cat #53

which are pictured indicate the relative increase in cisternal pressure and are representative records of the response to each type of solution administered Clearly the maximum elevation of pressure is seen in the distilled water record This is a consistent and reproducible finding The rise in pressure begins within the first minute of administration and is gradual and sustained throughout the drip which for the average animal occupied about 30 minutes in the dose of 40 cc /kg and at the rate of 60 drops per minute

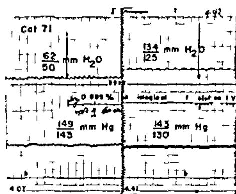
The blood cerebrospinal fluid and brain samples were obtained at the termination of the intravenous drip In 5 animals to which half strength saline was given and in 4 animals to which distilled water was given and on which chemical studies are complete there have been significant alterations in brain water and electrolyte content which will be reported

SUMMARY

A project to study brain swelling is introduced Control data is presented Preliminary methods which consistently produce elevated cerebrospinal fluid pressure by the *introduction of 445 per cent physiologic salt solution* and distilled water intravenously have not under the stated experimental conditions produced significant alterations of brain water or electrolyte content (sodium and potassium) This study is being systematically pursued and will constitute the basis of subsequent reports

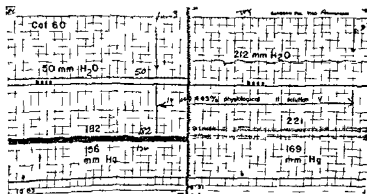
Table 1

DETERMINATION		EXPRESSED AS	NUMBER OF DETERMINATIONS (ANIMALS)	MEAN	STANDARD DEVIATION
Brain water gray		% wet weight	27	78 7	1 1%
Brain water white		% wet weight	27	67 6	1 4%
Serum sodium		mEq /liter	31	157 4	9 0 mEq
Serum potassium		mEq /liter	31	3 9	0.5 mEq
C.S F sodium		mEq /liter	28	157 8	6 9 mEq
C.S F potassium		mEq /liter	29	2 9	0.5 mEq
Brain gray	sodium	mEq /liter	11	61 8	9.3 mEq
	potassium	mEq /liter	11	75.5	9 6 mEq
Brain white	sodium	mEq /liter	11	49 0	11 0 mEq
	potassium	mEq /liter	11	65.5	6 0 mEq



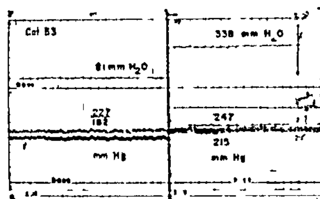
ADMINISTRATION of 0 889% PHYSIOLOGICAL SALT SOLUTION
Split record to demonstrate pre injection C S F pressure and terminal record at complet on of drip

Fig 2 Reproduction of original record cat #71



ADMINISTRATION of 0 445% PHYSIOLOGICAL SALT SOLUTION
Split record to demonstrate pre injection C S F pressure and terminal record at complet on of dr p

Fig 3 Reproduction of original record cat #60



ADMINISTRATION of DISTILLED WATER
Split record to demonstrate pre injection
C S F pressure and terminal record at
completion of drip

Fig 1 Reproduction of original record cat # 83

which are pictured indicate the relative increase in cisternal pressure and are representative records of the response to each type of solution administered Clearly the maximum elevation of pressure is seen in the distilled water record This is a consistent and reproducible finding The rise in pressure begins within the first minute of administration and is gradual and sustained throughout the drip which for the average animal occupied about 30 minutes in the dose of 10 cc /kg and at the rate of 60 drops per minute

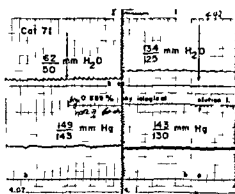
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SUMMARY

A project to study brain swelling is introduced Control data is presented Preliminary methods which consistently produce elevated cerebrospinal fluid pressure by the introduction of 445 per cent physiologic salt solution and distilled water intravenously have not under the stated experimental conditions produced significant alterations of brain water or electrolyte content (sodium and potassium) This study is being systematically pursued and will constitute the basis of subsequent reports

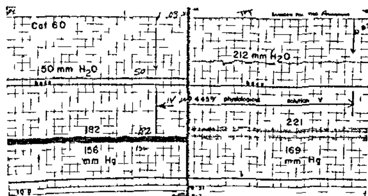
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Brain gray	sodium	mEq/liter	11	61.8	9.3 mEq
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Brain white	sodium	mEq/liter	11	49.0	11.0 mEq
	potassium	mEq/liter	11	65.5	6.0 mEq



ADMINISTRATION of 0.889% PHYSIOLOGICAL SALT SOLUTION
Split record to demonstrate pre injection C.S.F. pressure and terminal record at completion of drip

Fig. 2. Reproduction of original record cat #71



ADMINISTRATION of 0.445% PHYSIOLOGICAL SALT SOLUTION
Split record to demonstrate pre injection C.S.F. pressure and terminal record at completion of drip

Fig. 3. Reproduction of original record cat #60

intracranial pressure change Seven animals were sacrificed on the seventh postoperative day The remaining 16 were sacrificed at varying intervals up to 16 days

The dogs were sacrificed under light nembutal anesthesia using intra cardiac perfusion with physiologic saline followed by 10 per cent neutral formalin The injections were made under a pressure of 120 mm of mercury

The brain and spinal cord were transversely sectioned at 0 5 cm intervals Blocks were taken from the parasagittal and convex portions of each frontal lobe at the level of the anterior tip of the lateral ventricle from each fronto-parietal area immediately beneath each plug from each parieto-occipital junction from the middle thalamic section the first midbrain section the middle pontine section including cerebellum the second section of medulla including cerebellum and the lowermost section of available spinal cord

The first complete section of each paraffin block was cut at 5 μ and stained with hematoxylin and eosin The next 10 sections were cut at 20 μ and stained for Nissl substance following the method described by Windle *et al* Eight sections were then cut at 10 μ and stained both for myelin sheaths and axis cylinders using the Margolis modification of the Kluver Barrera stain In 3 experimental animals 150 serial Nissl stains were done using extra blocks of the medulla pons and midbrain

RESULTS

Macroscopic abnormalities were produced only in the 4 animals which died within 7 minutes to 16 hours as a result of the experiment These animals had been exposed to increases in intracranial pressure varying between 32 and 38 lb /sq in

In these animals there were large extravasations of blood in the subarachnoid space over the cerebral and cerebellar hemispheres and about the base of the brain Petechial hemorrhages were also present in the pons and midbrain in all 4 dogs and in 2 of these animals also in the medulla thalamus and the vermis of the cerebellum

Among the microscopic alterations the most important was the chromatolytic change observed in the medial and lateral reticular nuclei of the medulla pons and midbrain and to a lesser extent in some large cells in the tegmentum of the pons and midbrain The involved cells in these areas particularly at the 7 day interval were swollen the nuclei were oval in shape and eccentric in position and the Nissl substance was either completely lost or that which remained formed a thin rim of the periphery of the cell and also a small nuclear cap

While we have not investigated the time of onset this change was apparent as a perinuclear loss of Nissl substance by the third day Chromatolysis of this type was present in all of the experimental animals except the 4 which died within 16 hours 3 with minimal physiologic effects which were sacrificed between 35 and 12 days respectively and 1 with moderate physiologic effects which was sacrificed after 16 days

The animals exhibiting this type of chromatolysis had been exposed to increases in intracranial pressure varying between 3 and 36 lb /sq in The number of areas and number of cells within the areas exhibiting these changes appeared to be more directly related to the magnitude of the intracranial pressure increases than were the physiologic results In 5

ALTERATIONS IN CELL STRUCTURE FOLLOWING SUDDEN INCREASE IN INTRACRANIAL PRESSURE*

B F Haddad, J E Chason, H R Lissner, John E Webster and E S Gurdjian

Brain concussion is now generally regarded as having a histopathologic basis There is however, no uniform agreement as to the nature of change nor its location The variability of the reported pathologic change has been due, in part to the lack of a generally acceptable definition of concussion Experimental problems have been concerned with the physiologic criteria to be used to determine pure concussion in animals with methods of producing concussion and with adequate and comparable histologic methods of fixation and staining to permit uniform comparison of control and experimental material

In the following preliminary report emphasis has been placed upon the nature and location of the histologic changes accompanying sudden increases in intracranial pressure The method used to produce this increase in pressure has been described previously by Gurdjian *et al* and is similar to a method used by Walker *et al* to produce concussion It was hoped that the morphologic changes seen in concussion might be more clearly understood when produced by a carefully controlled method with but a single quantitatively variable factor

METHOD

Mongrel dogs weighing 5 to 15 kg were used This was done because a baseline for the physiologic effects in dogs had been determined previously with the same methods This series consisted of 8 control and 19 experimental animals

Each control and experimental animal was anesthetized using 1 ml of nembutal per 2 3 kg body weight With the head firmly fixed separate *single trephine openings* were made in the calvarium over the *left and right* frontoparietal areas parasagittally A plug for the delivery of the air pulse was screwed into place over the intact dura on the right side The dura on the left side was then opened and a strain gauge plug used to measure intracranial pressure and its duration was screwed into position in the subarachnoid space It was connected through a bridge to a cathode ray oscillograph which recorded the magnitude of the intracranial pressure it was also connected to an electronic synchronous clock which recorded the duration of pressure changes Both instruments were in connection with a second cathode ray oscillograph which superimposed both records into a single tracing which was then photographed

The blood pressure and pulse were recorded by means of a mercury manometer attached to the femoral artery while respirations were recorded by means of a chest tambour actuated by air

Each of the experimental animals was subjected to a single air pulse lasting for 0 01 ± 0 005 sec By changing the intensity of the air pulse intracranial pressures varying from 38 to 3 lb /sq in were produced

Four of the experimental animals died within 16 hours as a result of the

*From the *Wayne University Neurosurgical Service Grace Hospital Detroit Michigan*

EVIDENCE OF RESERVE IN THE INNERVATION OF THE URINARY BLADDER OF CATS: A PRELIMINARY STUDY DIRECTED AT IMPROVING THE THERAPEUTIC APPROACH TO BLADDER DYSFUNCTION IN MAN FOLLOWING CAUDA EQUINA INJURY*

A. YALE GEROL, R. JAMES SEYMOUR, ARTHUR A. PAVA,
JOSEPH W. ROBERTSON, SVEIN BOYESEN, DESMOND CALLAN
AND JAMES B. CAMPBELL

Clinicians handling the problem of neurogenic dysfunction of the bladder and urethra cannot state with assurance in every instance which component of the innervation is inadequate. This failure may occur because data applicable to chronic dysfunction in man are lacking, since most knowledge of bladder *innervation is based on results derived from stimulation* of acute cat preparations. According to Fulton[1] demonstration of physiologic function under these circumstances is limited because mechanisms normally responding in sequence are thrown simultaneously into activity. Therefore after ablation of selected sacral and coccygeal nerve roots observation and testing for periods ranging from 2 to 18 months were undertaken in female cats.

METHOD

Surgical. Each animal except the controls was submitted to limited resection of the components of the sacral and coccygeal nerve roots. All surgery was performed under aseptic conditions and full anesthesia, intravenous nembutal 20 to 30 mg./kg. Individual experiments were designed to demonstrate: 1. The contribution made to mictural function by either a dorsal or ventral division of any of the 3 pairs of sacral nerves existing in the cat, or the first 2 coccygeal nerves as a whole. 2. The minimum of these structures necessary for adequate micturition.

Postoperative Testing. The following methods were employed: 1. Clinical evidence of the ability to void. 2. Palpation for bladder tone and sphincter resistance during the expression of urine. 3. Continence judged by failure to lose urine when jounced in the upright position. 4. Excretory urography following intravenous injection of 35 per cent diodrast. 5. Cystometry following subcutaneous injection of bulbocapnine hydrochloride (Merck) 30 mg./kg.

This drug produces a catatonic-like state and permits cystometry *in the* unanesthetized animal. Carpenter and Root found that at comparable bladder volumes the intravesicle pressures were of the same order whether nembutal or bulbocapnine were used. A dampening effect was obtained when the chronic preparations to be reported were tested under light nembutal anesthesia. This diminished with the addition of bulbocapnine. Little is known about the pharmacology of this drug and any statement

*From the Department of Neurological Surgery, College of Physicians and Surgeons, Columbia University, New York City. Aided by grants from Department of the Army, Office of the Surgeon General (Contract #DA-49-007-MD 545), United Cerebral Palsy Associations Inc., Playtex Park Research Institute and the James Foundation.

animals which were exposed to intracranial pressures of 3 to 8 lb /sq in there were no physiological changes recorded The chromatolytic changes in these animals were limited to a very small proportion of the cells of the medial reticular substance in the medulla and pons

Similar chromatolytic changes were not present in the cells of the cerebral cortex even in the area directly under the air pulse The only change observed in this area was the irregular occurrence of an occasional slightly swollen or shrunken cell in which the Nissl substance appeared more finely granular than normal There were no changes in the cells of the cervical portion of the spinal cord

Definite alteration in the axis cylinders were prominent in only 2 of the experimental animals both of which died as a result of the sudden increase in intracranial pressure The changes consisted of fragmentation and swelling and were limited to fibers of the base and near the midline of the pons and medulla

Petechial hemorrhage other than those due to placement of the strain gauge plug and in those animals already mentioned rarely were found

No initial damage was recognized in the oligodendroglia cells the astrocytes or microglial cells Neuronophagia was occasionally noted in some of the more severely damaged cells The myelin sheaths were of normal appearance The lining cells of the ependyma and choroid plexus appeared unaltered in these animals There was no evidence of brain swelling

SUMMARY

1 A pressure pulse of varying magnitude but of uniform duration was applied to the unopened dural sac in the dog This produced physiologic effects of varying degrees Subsequently the brain was studied grossly and microscopically

2 Microscopic abnormalities were present in all but 4 of the experimental animals The changes were chromatolysis fragmentation of the axis cylinders and petechial hemorrhages.

3 The most significant change following a sudden increase in intracranial pressure in these dogs was chromatolysis involving cells in the reticular substance of the brain stem usually in a medial position with impacts of lesser magnitude and extending more laterally with impacts due to greater levels of pressure

role of the first sacral nerve are derived from cats in which either both first sacral nerves or a first ventral and the contralateral dorsal root are preserved The time needed for return of bladder function after operation varies inversely with the extent of the rhizotomy, and probably the degree of interruption of blood supply to the conus Ablation of the first 2 coccygeal or the 2nd and 3rd sacral nerves is followed by the greatest delay Increased bladder size remains for months after loss of the 2nd and 3rd sacral nerve roots Incontinence usually subsides at the end of the second postoperative week.

Nine types of preparations selected from a large group of operated animals for analytic consideration of the segmental innervation of the bladder are illustrated in Figure 1 The white components of the sacral and coccygeal nerve roots represent the anatomical deficit The volume of saline which provoked sufficient detrusor contraction to cause forceful expulsion of bladder contents around the catheter is recorded in cc above each diagram The box to the right in each case shows the relative bladder volume determined by repeated palpation after complete convalescence The arrow next to the box indicates the relative reduction in sphincter resistance detected during expression of urine

The data recorded in diagrams 1 2 3 5 and 6 show only minor deviations from the normal Preparation 1 had a definitely enlarged bladder compared with the smaller firm bladders of 3 and 6 Tonus judged by palpation was confirmed by cystometry Reduction in sphincter tone detected in cats 4 5 and 6 in which the coccygeal rhizotomy was added did not lead to incontinence All 6 preparations voided satisfactorily

Preparations 2 3 5 and 6 in which a single 2nd or 3rd sacral nerve root is preserved suggest that the coccygeal nerves aid in but are not essential to bladder function On the other hand micturition in preparation 1 is satisfactory when a single first ventral sacral root functions in conjunction with its contralateral dorsal component and the first 2 coccygeal nerves In the absence of the latter an opposing pair of first sacral nerves carry on only a fair degree of function as indicated in preparation 4

The coccygeal nerves probably play a supplementary role in micturition This is demonstrated by preparations 7 8 and 9 The bladder volumes in each instance were increased and bladder tone as well as sphincter resistance decreased These cats all voided in their cages as did the 6 others above and were continent by the standards of the experiment except for preparation 8 This was the only animal which lost urine in drops when jounced in the upright position On the other hand continuous overflow incontinence as is the case with an atonic bladder did not occur in this animal An accommodating intravesicle pressure curve rose abruptly to a level above 600 mm of water when the limit of bladder capacity was reached At this point bladder contents were forcibly expelled around the catheter in all cats except for preparation 8 In this case a 115 cc volume provoked expulsion at an intravesicle pressure of 300 mm of water The excellent clinical performance of this animal may have resulted from an adjustment in sphincter resistance and detrusor tone

The first 6 preparations indicate that sufficient motor impulses for micturition can reach the motor end organs in the bladder via any one of the 3 pairs of ventral sacral nerve roots Preservation of an individual

concerning its action on the detrusor muscle would be premature until further data are analyzed. Nevertheless, the pressure volume data that follow are of value because in all tests the conditions are constant except for the anatomical deficits. The procedure was carried out by introducing a #9 French double lumen ureteral catheter into the bladder via the urethra. A spinal manometer was connected with one lumen, and a 50 cc burette with the other. Zero pressure on the manometer was adjusted to the level of the symphysis pubis after draining the bladder. Saline was added continuously and readings made at regular intervals and whenever there was a significant rise in the manometer. The detrusor muscle of the non-operated cat expelled saline around the catheter when the volume reached 30 cc. At this point the intravesicle pressure rose above 600 mm. of water.

DISCUSSION

Production of reliable preparations for testing was hampered by coincidental ischemic damage to the sites of reflex mechanisms subserving micturition in the conus. Rhizotomy interrupts tributaries to the blood supply of this area. Variations in the vascular reserve of the individual animal may cause dissimilar results in a series when identical rhizotomies are performed. A rhizotomy which spares the coccygeal nerves is usually better tolerated than one which does not. Paraplegia and a downhill course frequently follow resection of all but one first sacral root even though the coccygeals remain intact. On the other hand, removal of all sacral and coccygeal nerves except for a unilateral second or third nerve is reasonably well tolerated, possibly because vessels passing in these roots permit adequate regional blood supply. Therefore, data concerning the

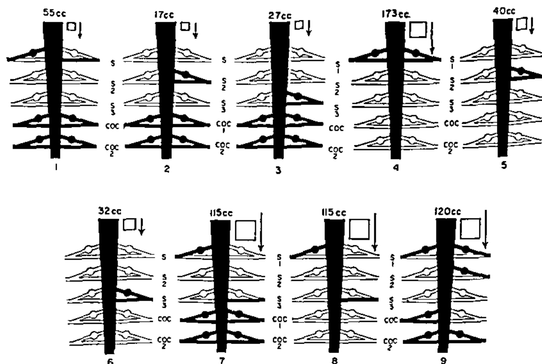


Fig. 1. These diagrams portray the dorsal and ventral components of the 3 sacral and first 2 coccygeal nerves as well as the spinal cord.

METHOD

General anesthesia with intravenous pentothal curare solution and endotracheal nitrous oxide and oxygen was used on all of the children and 5 of the 12 adults In the remaining 5 adults the anesthesia was limited to local infiltration with 1 per cent procaine An incision 3 to 4 cm long was made over the bicipital groove at the junction of the middle and distal thirds of the arm The segment of the brachial artery exposed at this level is distal to the origin of the profunda brachial and the superior ulnar collateral arteries The brachial fascia was incised and the neurovascular bundle identified The medial and ulnar nerves were retracted to expose the deeper lying brachial artery A 2 to 3 cm segment of the artery was mobilized and isolated by placing behind it a small rubber dam drain A needle with stylet was selected according to the size of the artery A ≠18 gauge spinal needle was used in the 6 month old child while a ±15 gauge needle was easily introduced in the older cases The needle was inserted in a proximal direction for 1 to 2 cm The stylet was left in place throughout the procedure except during the actual injection A small bulldog clamp was placed on the artery distal to the entrance of the needle to prevent peripheral flow of the contrast medium The clamp was removed between injections The amount of the contrast medium (35 per cent diodrast) used varied according to the size and age of the patient A volume of 8 cc was sufficient for adequate filling in the 6 month old child and 15 to 18 cc was used in the older children In the adults as much as 30 cc was injected but the average was approximately 20 cc The dye was injected as rapidly as possible to produce a bolus of diodrast traveling refluxly through the brachial into the subclavian artery When it reached the origin of the vertebral artery the bolus was carried with the normal blood flow into the vertebral basilar distribution Three radiographic exposures were made with each injection The first exposure was made just as the injection was completed the others at one second intervals thereafter The anteroposterior exposure was made with the x ray tube inclined 35 above a horizontal plane through the skull The needle puncture defect in the artery in children was closed with a single arterial suture of 5 0 silk in adults the arterial suture was not necessary because pressure for a few minutes with a sponge resulted in adequate closure of the needle hole The incision was closed with interrupted silk sutures and the arm was wrapped with an elastic bandage to prevent later formation of hematoma

With this technique both the vertebral and carotid systems are usually filled

Complications The only complication observed in this series of 32 patients was tingling in the fingers in 1 patient It was felt that this was due to the use of more than the usual traction on the nerves to expose the artery The tingling ceased upon relaxing the traction No neurological defect could be found after the procedure was completed No attempts have been made to inject the brachial artery percutaneously because it was felt that the danger of trauma to the median and ulnar nerves exceeded the disadvantages of a minor incision

The possibility of spasm occurring in the brachial artery has been considered but was not seen in any of these cases Radner[4] and Hauge[1]

second or third sacral nerve root leads to more successful performance than is the case with the first. The importance of the dorsal root system in bladder physiology is demonstrated by preparations 1, 7 and 9. The dorsal sacral roots may function as "primers" in Lassek's[3] terminology. He showed that 1 remaining major dorsal root in the cervical plexus of a monkey permitted voluntary movement of the arm without loss of tone. A similar function may be obtained in the plexuses of the bladder and urethra.

Short term observation of animals in which total ablation of the sacral roots has been carried out in stages suggest that adequate micturition may be possible under these conditions.

In all types of preparations intravenous urography failed to demonstrate persisting uretero- or pyelo-ectasia but confirmed clinical progression of bladder size.

CONCLUSION

These observations demonstrate a hitherto unsuspected reserve in the innervation of the bladder of the cat.

REFERENCES

1. Fulton, J. F.: Physiology of the Nervous System. Third Edition. New York, Oxford University Press, 1949, pp. 141-142.
2. Carpenter, F. G., and Root, W. S.: Effect of parasympathetic denervation on feline bladder function. Am. J. Physiol., 1951, *166*: 686-691.
3. Lassek, A. M.: Inactivation of voluntary motor function following rhizotomy. J. Neuropath., 1953, *12*: 83-87.

VERTEBRAL ANGIOGRAPHY BY RETROGRADE INJECTION OF THE BRACHIAL ARTERY*

PURDUE L. GOULD, WILLIAM T. PEYTON AND LYLE A. FRENCH

Vertebral angiography has become of sufficient value in the diagnosis of lesions of the posterior fossa to warrant exploring new methods to accomplish the procedure. The technique of filling the vertebral system has been described by many authors (Moniz 1933,[3] Schimidzu 1937,[6] Sjoquist 1938,[7] Takahashi 1940,[9] Sugar 1949,[8] Lindgren 1950, Radner 1951,[4] Sergent 1952,[5] and Hauge 1954[1]) but these techniques are designed primarily for use in adults and are difficult to perform in children.

The purpose of this report is to present our experience in a series of 32 cases in whom it was attempted to fill the intracranial vascular tree by retrograde brachial artery injection. Since 1952 it has been carried out upon 12 adults and 20 children ranging in age from 6 months to 65 years. Successful filling of the posterior fossa circulation was obtained in 8 of the 12 adults and in all of the children. The 4 adult cases classified as unsuccessful showed some degree of contrast medium in the vessels but it was considered insufficient for diagnostic purposes.

* From the Division of Neurosurgery, Department of Surgery, University of Minnesota Medical School, Minneapolis, Minnesota.

METHOD

General anesthesia with intravenous pentothal-curare solution and endotracheal nitrous oxide and oxygen was used on all of the children and 5 of the 12 adults In the remaining 5 adults the anesthesia was limited to local infiltration with 1 per cent procaine An incision 3 to 4 cm long was made over the bicipital groove at the junction of the middle and distal thirds of the arm The segment of the brachial artery exposed at this level is distal to the origin of the profunda brachial and the superior ulnar collateral arteries The brachial fascia was incised and the neurovascular bundle identified The medial and ulnar nerves were retracted to expose the deeper lying brachial artery A 2 to 3 cm segment of the artery was mobilized and isolated by placing behind it a small rubber dam drain A needle with stylet was selected according to the size of the artery A #18 gauge spinal needle was used in the 6 month old child while a #15 gauge needle was easily introduced in the older cases The needle was inserted in a proximal direction for 1 to 2 cm The stylet was left in place throughout the procedure except during the actual injection A small bulldog clamp was placed on the artery distal to the entrance of the needle to prevent peripheral flow of the contrast medium The clamp was removed between injections The amount of the contrast medium (35 per cent diodrast) used varied according to the size and age of the patient A volume of 8 cc was sufficient for adequate filling in the 6 month old child and 15 to 18 cc was used in the older children In the adults as much as 30 cc was injected but the average was approximately 20 cc The dye was injected as rapidly as possible to produce a bolus of diodrast traveling refluxly through the brachial into the subclavian artery When it reached the origin of the vertebral artery the bolus was carried with the normal blood flow into the vertebral basilar distribution Three radiographic exposures were made with each injection The first exposure was made just as the injection was completed the others at one second intervals thereafter The anteroposterior exposure was made with the x ray tube inclined 35° above a horizontal plane through the skull The needle puncture defect in the artery in children was closed with a single arterial suture of 5 0 silk, in adults the arterial suture was not necessary because pressure for a few minutes with a sponge resulted in adequate closure of the needle hole The incision was closed with interrupted silk sutures and the arm was wrapped with an elastic bandage to prevent later formation of hematoma

With this technique both the vertebral and carotid systems are usually filled

Complications The only complication observed in this series of 32 patients was tingling in the fingers in 1 patient It was felt that this was due to the use of more than the usual traction on the nerves to expose the artery The tingling ceased upon relaxing the traction No neurological defect could be found after the procedure was completed No attempts have been made to inject the brachial artery percutaneously because it was felt that the danger of trauma to the median and ulnar nerves exceeded the disadvantages of a minor incision

The possibility of spasm occurring in the brachial artery has been considered but was not seen in any of these cases Radner[4] and Hauge[1]



Fig. 1 Right retrograde brachial angiogram Left common carotid artery compressed in the neck Note filling of both internal carotid systems and the vertebral circulation



Fig. 2 Right retrograde brachial angiogram demonstrating vertebral and internal carotid circulation

report that this complication usually occurs when the catheter reaches the subclavian artery Radner[4] advises that a 2 5 per cent solution of papaverine hydrochloride be administered 1 hour before injection to prevent spasm from occurring In none of our cases was medication employed to prevent spasm since the needle was introduced only 1 to 2 cm into the brachial artery

The site of the exposure was selected with the possibility that thrombosis might occur Since the exposure is distal to the major collateral arteries the circulation should be entirely adequate if thrombosis occurs In all of our cases radial arterial pulsations were easily palpated following removal of the needle

The use of a stylet obviates the need for continuous saline in the interval between injections The volume of saline needed to maintain patency of the needle lumen might overload an infant's circulation

No case exhibited any reaction from the injection of diodrast other than the usual flushing sensation similar to that seen during carotid angiography One adult received a total of 175 cc of 35 per cent diodrast in 5 injections in an attempt to obtain adequate filling

DISCUSSION

This procedure has proven quite successful in children In addition to the vertebral filling there has been excellent visualization of the ipsilateral internal carotid circulation in all the children except one On the anteroposterior projection contralateral compression of the carotid artery in the neck improves the ipsilateral filling of the carotid system and may lead to adequate visualization of the distribution of both internal carotid arteries The filling of the internal carotid circulation has been sufficient with this technique to justify its use for investigating lesions in younger children in whom percutaneous puncture might be difficult Three cases have been

performed on the left side with bilateral carotid compression Filling of the internal carotid circulation through the posterior communicating arteries resulted in all three

The results with this technique in adults have not been encouraging The adult arteries are proportionately larger and consequently the greater quantity of blood dilutes the bolus of contrast medium In order to overcome this dilution factor it was necessary to inject excessively large amounts of dye The possible risk with the use of such large dosages makes this technique in adults less feasible than percutaneous vertebral artery puncture

CONCLUSION

A technique for performing vertebral angiography has been described It is recommended for use in children in whom it is desired to investigate the vertebral or internal carotid circulation It is not recommended for use in adults except where other attempts at vertebral angiography by the usual methods have failed

REFERENCES

1 Hauge T Catheter vertebral angiography Acta radiol Suppl Stockh *109* 1 219 1954
2 Lingren E Percutaneous angiography of the vertebral artery Acta radiol Stockh *33* 389-400 1950
3 Moniz E and Alves A L importance diagnostique de l arteriographie de la fossa posterieure Rev neur Par 2 91 96 1933
4 Radner S Vertebral angiography by catherization A new method employed in 221 cases Acta radiol Suppl Stockh *87* 134 1951
5 Sergent P Rougerie J Pertuiset B and Petit Dutaillis D L angiographie vertebrale percutanee cervicale anterieure d apres 130 cas Technique et bases de l interpretation des cliches Prat méd fr *60* 1415 1418 1952
6 Schmidzu K Beitrage zur arteriographie des gehirns—einfache percutane methods Arch klin Chir *188* 295 316 1937
7 Sjoquist O Arteriographische darstellung der gefusse der hintern schadelgrube Chirurg *10* 337 380 1938
8 Sugar O Holden L B and Powell C B Vertebral angiography Am J Roentg *61* 166 182 1949
9 Takahashi K Die percutane arteriographie der arteria vertebralis und ihrer versorgungsgebiete Arch Psychiat Berl *111* 373 379 1940

ELECTROENCEPHALOGRAPHIC RESPONSES IN DOGS DURING REDUCED BLOOD FLOW*

GUY OWENS, JOHN L. SAWYERS AND JAMES W. WARD

Electroencephalographic responses during low rates of blood flow have not been reported. Azygos rates of flow have been taken previously as a standard measurement in low blood flow experiments.[1] In the experiments to be reported electroencephalographic tracings were studied in dogs during low rates of flow (as judged by this technique) under normal and reduced body temperatures.

METHOD

Under intravenous pentobarbital anesthesia (30 mg./kg.) and endotracheal intubation 21 healthy mongrel dogs weighing between 6 and 20 kg. were subjected to right thoracotomies through the 4th interspace. Lung insufflation was maintained by a demand type respirator using oxygen in 3 operations and compressed air for the rest. No variations were observed. Occluding tapes were passed around the superior and inferior venae cavae and around the azygos vein. Low blood flows were produced by occlusion of the venae cavae alone. Almost instantaneous venous inflow stasis was produced by occluding the venae cavae and azygos vein. Continuous bipolar electroencephalographic and transthoracic electrocardiographic tracings were made throughout the experiments. Two needle electrodes symmetrically embedded in the skull contacted the cerebral cortex in the posterior parietal regions and an indifferent electrode was attached to the left ear. A 4 channel Offner Electroencephalograph was used.

Hypothermia ranging between 25 and 28°C. was produced in 11 animals immersed in an ice water bath. These animals were rewarmed by immersion in hot water.

Cannulation of the azygos vein and measurement of blood collected for a 20 second period during venae cavae occlusion was carried out in all animals surviving the low rates of flow. In 5 of these animals rates of azygos flow were also measured by the method described by Cohen and Lillehei.[1]

The blood pressure was recorded from the femoral artery. Cerebrospinal fluid pressures were measured by a water manometer through a needle in the cisterna magna. Microscopic studies were made of the brain and the lung in those animals which expired or demonstrated neurological deficits. All survivors were followed for one month and then sacrificed.

RESULTS

In Table 1 results of studies on 20 animals during azygos flows are summarized. At normal temperatures the appearance of a flat electroencephalogram first detected azygos flows of less than 10 cc./kg./min. (Fig. 1). Cardiac irregularities usually followed the isoelectric responses within 2 to 4 minutes. All animals died though at varying intervals in which venous return was not fully restored within 2 minutes of the development of a flat tracing. In those animals in which the rate of azygos flow was measured at 10 to 15 cc./kg./min. slight electroencephalographic slowing

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Table I Electroencephalographic Responses in 20 Animals During Azygos Flow Studies

Normal Temperature

Dog Number	Azygos Flow cc/kg/min	Min Azygos Flow Only	Final EEG change During Azygos Flow	B P During Azygos Flow	CSF press (mm H_2O) During Azygos Flow	Final Result
687	2 6	31	Decreased amplitude			Died PO 2 (technical error)
693	4 7	12	Isoelectric			Sacrificed PO 17 (dog blind)
694		20	Isoelectric			Ventricular fibrillation
585	2 7	20	Isoelectric			Ventricular fibrillation as chest was being closed
722	10 6	60	Moderate slowing			Died PO 2 hemorrhagic atelectasis
724	13 4	60	Moderate slowing	25		Died PO 1 hemorrhagic atelectasis
808	13 4	30	No change	20	-8	Survived
829	6.0	25	Isoelectric	20	120→90	Died PO 1 hemorrhagic atelectasis
895	7 5	10	Isoelectric	20	120→50	Survived
900	12 5	30	No change	20	100	Survived
Hypothermia (25° 28°C)						
719	5 4	60	Intermittent isoelectric periods	60		Died immediately post-op (technical error)
730	3 2	30	Marked slowing	25		Died 15 hr post op hemorrhagic atelectasis
736	6 6	40	Isoelectric	25		Survived
742	2 8	60	Moderate slowing decreased amplitude	30		Died PO 2 hemorrhagic atelectasis
253	1 3	30	Moderate slowing decreased amplitude	18		Survived
767	1 01	30	Moderate slowing decreased amplitude			Survived
785	3 1	30	Isoelectric		110→50	Survived
872	6 3	30	No change			Survived
874	3 5	30	No change	20	50	Survived
883	5 4	30	Moderate slowing decreased amplitude	20	85	Survived

and amplitude increases were noted In 2 of these animals the electroencephalographic changes persisted during a period of 60 minutes on such circulatory levels

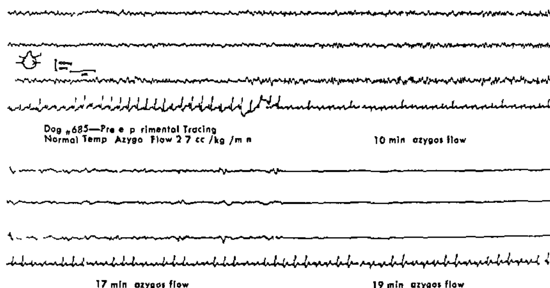


Fig 1 Electroencephalographic and electrocardiographic tracings from dog #685 This record illustrates the response to azygos flows less than 10 cc /kg /min at normal temperatures

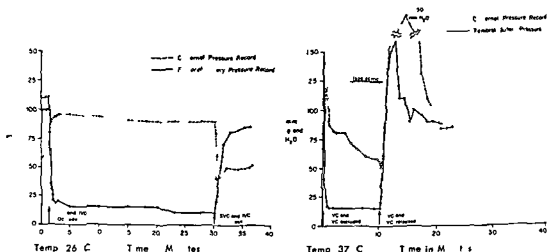


Fig 2 Representative reactions of blood pressure cerebrospinal fluid pressure and electroencephalograms in animals at normal and low body temperatures during venae cavae occlusion

At temperatures between 25 to 28 C rates of flow ranged from 1 8 to 6 8 cc /kg /min and were tolerated in 8 instances for 30 minutes with only moderate electroencephalographic changes Two of these animals were studied for 60 minutes without noticeable change during the second half hour In 2 other cases results differed In animal #736 waves of 2 to 3/sec occurring in spindles were observed at 30 minutes At 40 minutes the record had become isoelectric In animal #785 electroencephalographic activity disappeared at 25 minutes Azygos flow was continued for 5 minutes longer without evidence of cardiac irregularities

At normal temperatures with total venous inflow stasis electrical activity disappeared within 50 to 90 seconds and was followed by cardiac disturbances within 4 minutes At temperatures of 25 to 28 C with similar venous stasis the record became flat in around 3 minutes In dog #863 maintained

at 25 to 28°C 6 minutes of inflow stasis was followed by 5 minutes of azygos flow (measured at 2 7 cc /kg /min) Electrical activity disappeared after 2½ minutes of venous stasis Full venous return was then re established after 11 minutes and electrical activity reappeared after 30 minutes of total absence This animal survived without evidence of neurologic damage The electroencephalogram returned to normal In other experiments[2] after 10 minutes of venous inflow stasis during hypothermia survival rates fell precipitously Deaths were attributed to cardiac irregularities rather than neurological damage

Microscopic studies of the brain failed to reveal pathologic changes Hemorrhagic atelectasis was observed in the 3 animals who were carried for 60 minutes on azygos flow and who died from 21 to 36 hours postoperatively

Sequelae appeared in only one of the long term surviving animals Bilateral corneal opacities producing blindness developed in animal #693

DISCUSSION

The low arterial pressures remained remarkably similar in the various experiments despite differences in the venous flow and the temperature levels employed Figure 2 contrasts the responses of the cerebrospinal fluid pressures in 2 experiments These patterns are representative reactions The electroencephalogram did not become flat for a period of 30 minutes at the temperature levels used if the cerebrospinal fluid pressure remained at or near that reached immediately after venae cavae occlusion With a fall of cerebrospinal fluid pressure however the electrical activity disappeared within this period In the presence of hypotension the cerebrospinal fluid pressure normally adjusts to lower levels In the circumstances of this experiment it is suggested that because of the collateral cerebral venous return via the azygos vein slightly higher cerebrospinal fluid pressure despite severe hypotension is in effect a measurement of an increased venous pressure A fall in the cerebrospinal fluid pressure with unchanged arterial recordings could thereby indicate the effective development of a more adequate collateral venous return Since the disappearance of electroencephalographic activity even in the presence of hypothermia coincided with a drop in cerebrospinal fluid pressure one possible explanation is advanced that elevated venous pressure in the presence of hypotension is important in the maintenance of the electroencephalogram

Hypothermic dogs and those at normal temperature during 60 minutes of azygos flow alone developed mild transitory electroencephalographic changes but died 21 to 36 hours later Pathologic findings were limited to the lungs which showed hemorrhagic atelectasis with edema Noell and Chinn[3] observed similar electroencephalographic and pathologic changes in rabbits following repetitive anoxic periods produced by nitrogen inhalation It appears then that the presence of only mild temporary changes in the electroencephalogram during prolonged periods of altered metabolism does not reflect the animals ability to survive

The electroencephalogram during hypothermia is an inadequate signpost in accurately indicating the physiologic status of the animal In the presence of the low rates of blood flow and hypothermia a flat electroencephalogram was tolerated for undetermined periods whereas at normal body temperatures a definite tolerance was demonstrated Clowes and his co workers have

also demonstrated the importance of electroencephalographic tracings at normal temperatures[4]

SUMMARY

Ten dogs at normal and 11 at low body temperatures were studied by means of the *electroencephalogram electrocardiogram cerebrospinal fluid* and arterial pressures during and after low rates of blood flow Clinical and pathologic changes were recorded Marked differences in rates of azygos flow in different experiments at normal temperatures were reflected in changes in the electroencephalogram Variations in azygos flow were less marked at low body temperatures but the rate of flow was greatly reduced The electroencephalogram was found to be ineffective in evaluating the state of viability of the hypothermic animal during low rates of blood flow This was in contrast to its effectiveness at normal temperatures Increased tolerance of total ischemia as measured by the electroencephalogram during hypothermia was observed The relationship of elevated cerebral venous pressure in the presence of hypotension and the electroencephalogram are discussed

REFERENCES

1 Cohen M and Lillehei C W A quantitative study of the azygos factor during vena caval occlusion in the dog Surg Gyn Obst *98* 225 1954
2 Scott H W Jr Collins H A and Foster J H Hypothermia as an adjuvant in cardiovascular surgery experimental and clinical observations Am Surgeon *20* 799 812 1954
3 Noell W and Chinn H I The cerebral survival time of rabbits in anoxia Project No 21 02 071 School of Aviation Medicine Nov 1948
4 Clowes G H A Jr Neville W E Hopkins Amos Anzola J and Simeone F A Factors contributing to success or failure in the use of a pump oxygenator for complete by pass of the heart and lung experimental and clinical Surgery *36* 557 1954

AN ANALYSIS OF THE RESULTS OF TREATMENT OF INTRACRANIAL ANEURYSMS BY COMMON CAROTID ARTERY LIGATION*

NICHOLAS WETZEL AND RICHARD A DAVIS

Recently there has developed an opinion that ligation of the common carotid artery is a dangerous procedure of little value in the treatment of intracranial aneurysms and that a direct attack on these lesions is necessary and desirable The hazards of carotid ligation have been stressed by the general surgeon and its results well documented especially in regard to surgery of malignancies of the head and neck

METHOD

It has been our opinion that common carotid artery ligation has been an effective method of dealing with intracranial aneurysms and arteriovenous fistulas and that it has not been unduly hazardous The records of 104 patients with common carotid artery ligations from the Department of Surgery of Northwestern University Medical School have been studied

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These include 73 patients with verified or suspected intracranial aneurysms, 21 patients with various vascular malformations such as angiomas and arteriovenous fistulas, and a miscellaneous group of 7 cases which included a carotid body tumor and a cervical carotid aneurysm, all of which had an indication for a carotid artery ligation. Because only recently has cerebral angiography in our hands become an entirely satisfactory procedure, some of the earlier examples of these lesions were not confirmed angiographically, the diagnosis being made on the basis of symptoms and clinical examination.

In this series there were 58 males and 16 females; the ages ranged from 9 to 73 years, with 82 patients being between the ages of 20 and 60 years and 29 being between 21 and 30 years. Common carotid artery ligation in continuity with braided silk was done in 81 patients, ligation of the internal carotid artery in 6, and of both vessels in 15 patients, with division of the common carotid artery in one patient. The procedure was performed under local anesthesia in 81 patients, general anesthesia in 20.

The present status of these patients is as follows: known to be dead, 27; of these 11 died in the immediate postoperative period and 7 have died of a progression or recurrence of their disease process. 6 patients died of unrelated causes. Of this latter group one patient was shot to death by a hold-up man 1 months following ligation, one whose autopsy showed no evidence of recurrence of bleeding; one with an intracranial aneurysm succumbed to polycystic disease of the kidneys 2 years postoperatively; 2 died of cardiac failure and 2 of causes unknown but, as far as could be ascertained, not due to recurrence of intracranial bleeding.

Sixty-seven patients are alive. Of those living, 3 became hemiplegic following the ligation but otherwise have continued well. One of this group had one episode of recurrent bleeding, and it was thought that his ligature had loosened. He was operated upon again and has since been well more than 5 years; he is not hemiplegic although occasionally he has a seizure. The duration of follow-up of the remainder was as follows: 1 patient 22 years, 2 16 years, 1 13 years, 3 10 years, 2 9 years, 1 8 years, 5 7 years, 15 6 years, 9 5 years, 1 4 years, 4, 3 years, 8 2 years and 6 1 year or less.

There were 61 patients who sustained no increase in their neurologic deficit following ligation. Of this group 51 were in good general condition at the time of the ligation while 8 patients were considered to be in poor condition, either semistuporous (1), stuporous (3) or comatose (1). These 8 patients all improved to the extent that they are now living and well. At the time of ligation 1 patient was 19 years of age and has been followed for 5 years. During this period she has borne children and has never had a recurrence of her initial subarachnoid hemorrhage. Another patient, 22 years of age, has been followed for 1 years. One patient 28 years of age has been followed for 6 years. One patient 29 years of age at the time of ligation has been seen regularly for 5 years. It may be added that all patients in this group have been traced adequately. In this group of 62 patients there were 18 who had varying degrees of hemiparesis prior to ligation. In none did the weakness become worse, but in 1 cases it persisted so as to become a definite defect. Three of these patients were stuporous at the time of ligation.

There were 3 patients in whom a definite postoperative hemiplegia occurred and all had angiographically verified intracranial aneurysms. The

age of 2 patients was 25 years and the third was 53 years the 2 youths had minimal neurological findings and were alert the older man had a blood pressure of 210/110 and was hemiparetic and stuporous One of the 25 year old patients had ligation performed under local anesthesia, and 18 hours postsurgically became hemiparetic and aphasic He seemed to improve with a stellate ganglion infiltration and accordingly a stellate ganglionectomy was performed followed by gradual improvement The other 25 year old patient had endotracheal anesthesia and the common carotid artery was divided because of the suggestion of Lambert Rogers that to prevent postoperative difficulties it was wiser to divide the vessel This patient became hemiplegic immediately but slowly showed signs of recovery of function and was subsequently discharged ambulant This was the only instance in this series in which division of the carotid artery was performed The 53 year old patient was obviously in poor neurological condition at the time of surgery, an open arteriogram was performed, following which the common carotid artery was ligated, the entire procedure was done under local anesthesia During the immediate postoperative period the hemiparesis became a hemiplegia During the 3 weeks prior to discharge he showed definite improvement was known to be alive 2 years ago and was economically and socially independent

It would seem that there were no common factors present to account for the hemiplegias which were not applicable to other patients in the series the patient in whom the carotid artery was divided perhaps is the single exception The combination of the general anesthesia plus the division of the artery may have been important factors in this one patient In another there was a marked hypertension Two of the patients had open arteriograms one had a percutaneous study one had ligation performed immediately following arteriography, and the others at varying times

There were 14 postoperative hospital deaths At the time of ligation 10 patients were considered to be terminal, 3 in poor condition and only 1 in reasonably good general condition Their ages ranged from 23 through 60 years with the greatest number (5 cases) in the age group between 30 and 40 years 4 were between the ages of 50 and 60 while others were scattered One patient was considered to be normal neurologically while 10 were definitely hemiplegic and 3 were decerebrate Local anesthesia was used in 8 patients with general anesthesia in the remaining 6 cases The common carotid artery was ligated in 11 patients the internal in 2 and both vessels in the remaining patient In 11 patients the lesion was an intracranial aneurysm In one the first manifestation of an intracranial tumor was a subarachnoid hemorrhage and in another patient a carotid ligation was performed for a subarachnoid hemorrhage the cause of which was found at autopsy to be a basilar fracture Two patients in this group had normal blood pressures 8 had a systolic pressure over 110 mm of Hg and in 3, the systolic blood pressure was over 190 mm

Essentially, the common factor in this group would seem to be an aneurysm hypertension and poor general or neurological status in only 1 of these patients was the general condition considered to be good but he was a hypertensive Ligation in this latter instance was performed under local anesthesia, and his progress was excellent for 4 days when suddenly he complained of headache became quadriplegic his systolic pressure rose

precipitately and he expired An autopsy was not granted but it must be assumed that he had another episode of subarachnoid bleeding The greater percentage of the patients who expired in the immediate postoperative period were apparently doomed at the time of surgery and probably little or nothing was achieved by the ligation

However, there were several patients in the group now alive who were in as grave condition at the time of ligation and who were not expected to survive In a few of these patients there was probably not a proper appreciation of the serious nature of the underlying pathology because several had their fatal episode of subarachnoid hemorrhage while awaiting ligation or diagnostic studies In this series there are only 3 known instances of recurrence of bleeding following ligation one occurred 4 days post ligation another had a minimal episode several months after ligation which has not recurred in the last 5 years a third patient had a fatal recurrent hemorrhage from an arteriovenous fistula 4 years after ligation Others have written of evacuating the intracortical hematoma which certainly must occur with aneurysmal bleeding but autopsy examination has proved to us that in many patients the bleeding was not only intracortical but intraventricular as well with a resulting respiratory collapse which would make any surgical approach futile

There were 7 patients in whom progression of the disease process occurred and of these 5 had an intracranial aneurysm 1 an angioma and 1 an arteriovenous fistula One patient had 2 aneurysms of the internal carotid artery which were verified angiographically At the time of ligation she was stuporous had a left hemiparesis a right 3rd 4th and 5th nerve lesion and a bloody cerebrospinal fluid She improved after ligation and was apparently well until she died of recurrence of her bleeding 4½ years after ligation of her common carotid artery Another patient was 67 years old had severe headaches a 5th nerve lesion and an angiographically demonstrated aneurysm of the left middle cerebral artery After common carotid ligation she improved but later developed further cranial nerve signs and died 3 months later A 47 year old man with multiple cranial nerve signs had a craniotomy at which time the necrotic temporal lobe was removed and later he developed neurological signs suggestive of an aneurysm Arteriography revealed an aneurysmal dilatation of the internal carotid artery Following ligation of the common carotid artery under endotracheal anesthesia he became hemiplegic and aphasic there was no improvement in the postoperative course and he died 3 months following surgery One patient with a large vascular anomaly presumably an angioma of the left parieto-occipital lobe was not improved following ligation in 1933 and died 1 year later after her discharge Another patient had survived 4½ years following ligation but died of a subarachnoid hemorrhage secondary to a ruptured arteriovenous fistula during the 9th month of pregnancy

In this latter group it might have been possible to achieve a better result with other forms of therapy but certainly a trial of conservative ligation was indicated In the 67 year old patient a more extensive surgical procedure such as clipping the aneurysm was certainly not indicated The patient with multiple aneurysms was improved and apparently well for 4 years The 47 year old male had probably had a previous rupture of the

dilated internal carotid artery into the temporal lobe and might have received benefit from a so called trapping operation The patient with the occipital lobe angioma, might have received more benefit from a lobectomy but such was not done in 1933 The patient with the post traumatic arterio venous fistula made good progress and one can speculate as to the influence the pregnancy had on her intracranial blood pressure

CONCLUSIONS

In conclusion it is our impression that carotid ligation is an effective form of therapy of intracranial vascular disease In the 73 patients with aneu rysms we have a verified recurrence in only 2 patients To this group could be added several more patients who were alive and well until they expired of some other cause Common carotid artery ligation was not a successful form of therapy in 14 patients of whom 12 were in poor condition or ter minal at the time of ligation It is questionable in our minds if anything could have aided the majority of those patients Definite postoperative hemiplegia occurred in 3 patients

We believe that ligation of the common carotid artery should be done with a local anesthetic and with the patient in as good general condition as is possible Ligation of the common carotid artery is technically easier than internal carotid artery ligation and the work of Sweet[1] has shown that it is as effective in reducing intracranial arterial pressure Since a consider able number of these patients will improve without surgical intervention the least harmful form of treatment is indicated We do not advocate cortical resection for the patient with an angioma whose only symptom might be seizures and/or subarachnoid bleeding Carotid ligation may well prevent recurrent bleeding and has not in our hands produced hemiplegia in this type of lesion in contrast to the experience of Olivecrona[3]

REFERENCES

1 Bakay L and Sweet W H Cervical and intracranial intra arterial pressures with and without vascular occlusion Surg Gyn Obs *95* 67 75 1952
2 Moore O and Baker H W Carotid artery ligation in surgery of the head and neck Cancer Phila *8* 4 712 726 1955
3 Olivecrona H and Riives J Arteriovenous aneurysms of the brain their diagnosis and treatment Arch Neurol Psychiat Chic *59* 567 603 1948
4 Rogers L Carotid ligation for intracranial aneurysm report of a case studied by electroencephalography Brit J Surg *32* 309 311 1944

THE TRICEPS REFLEX*

FRANK P SMITH

Increased interest in the diagnosis of herniated cervical intervertebral disc has focused attention on the importance of the triceps reflex This reflex holds the same relative position in a case of cervico brachial pain that the ankle jerk has in a case of sciatica A common site of cervical cartilage rupture is at the C6 disc level,[1] which causes compression of the C7 spinal nerve root Weakness atrophy and areflexia of the triceps muscle may be present in advanced cases of C7 root compression but early or partial lesions may express themselves in a more subtle manner

The segmental innervation of the triceps muscle has not been heretofore resolved for differential nerve root evaluation Also the classical method[2] of eliciting the tricep reflex presents certain technical difficulties that often defeat proper evaluation of the reflex

The purposes of this paper are

(1) to indicate the differential innervation of the components of the triceps muscle and

(2) to emphasize a method of eliciting the triceps reflex

METHOD

The three components of the triceps muscle are known as the long medial and lateral heads which have a common tendon for insertion Anatomical texts[4,5] list the triceps muscle innervation provided by the radial nerve arising from the C6 7 and 8 spinal nerve roots With neuroanatomical dissections in cadavers the author has found that the innervation of the long head of the triceps comes from the C7 root whereas the medial and lateral heads have a combination of innervation from the C7 and C8 roots This has been checked and confirmed by Dr Charles Tobin (Department of Anatomy University of Rochester Medical School) to whom the author is grateful An infant cadaver specimen prepared by Dr Tobin is shown in Figure 1 In the infant cadaver the nerves are large in relation to other structures and lend themselves to more accurate dissection

Thus from the neuroanatomical information gained the long head of the triceps muscle forms an isolated testing area for lesion of the C7 root It then becomes important to test the triceps reflex in a manner that a defect of the long head may be noted

According to the classical method of eliciting the triceps muscle the examiner stands in front of or to the side of the patient and strikes the tendon rather blindly A response may be obtained from the medial and lateral heads and a defect of the long head may not be noted Also in the classical method the muscle may be in a state of undue tension or inordinately opposed by the biceps muscle These alone may give misleading information The examiner finds it awkward hitting the right sided tendon if he is right handed and vice versa if he is left handed He is in a poor position to compare the response of one side with the other

A more favorable and rarely mentioned method for evaluating the triceps

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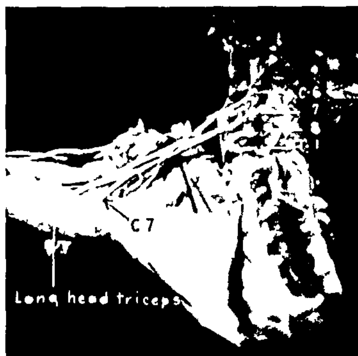


Fig 1 Showing innervation of long head of triceps muscle arising from C 7 spinal root

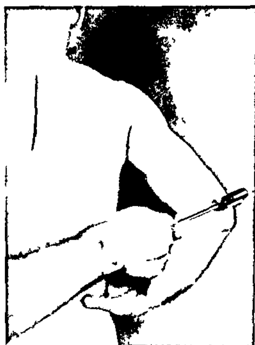


Fig 2 Elicitation of triceps reflex

reflex consists of having the patient place both hands on his hips (akimbo) [6] while the examiner stands behind him In this position of the upper extremities there is slight tension on the triceps tendon and avoidance of undue opposition by the biceps muscle Also in this position simple inspection may be of diagnostic help The long head of the triceps muscle normally produces a convex contour over the dorsum of the arm Partial atrophy which might not be apparent otherwise may be indicated by flattening of the convexity

As shown in Figure 2 the examiner has a full view for striking the triceps tendon and observing which components of the muscle respond to the stim

ulus The examiner is in a good position for comparing the response of one side with the other since he can tap the tendons alternately without changing his station The author has found that if the reflexes tend to be hypoactive palpation over the long head of the triceps during reflex testing may detect a difference in response that may not be grossly visible In the classical method the examiner would not have one hand free for palpation of the muscle

DISCUSSION

It is hoped that consideration of the segmental innervation of the triceps muscle and elicitation of the reflex as discussed above will assist in evaluating the multiple cases of cervicobrachial pain and other neurological disorders Surely the triceps reflex deserves a rightful place in the neurological examination rather than being skipped over lightly as often has been the case

SUMMARY

Factors of the triceps reflex have been presented relative to
(1) the differential innervation of the muscle components and
(2) elicitation of the reflex by the method reviewed above

REFERENCES

1 Bradford F K and Spurling R G The Intervertebral Disc. Springfield Illinois Chas C. Thomas 1947 p 125
2 Ibid p 125
3 DeJong R N The Neurologic Examination New York Paul B Hoeber Inc., 1950 p 563
4 Schaeffer J P Morris Human Anatomy New York Blakiston Co 1953 p 441
5 Johnston T B and Whillis J Gray's Anatomy London Longmans Green and Co., 1949 p 608
6 Wartenberg Robert Diagnostic Tests in Neurology Chicago Year Book Publishers Inc 1953 p 134

THE USE OF PLASTIC AND RUBBER TUBING IN THE MANAGEMENT OF IRREPARABLE NERVE INJURIES*

RICHARD WILLIAM GARRITY

Multitudinous methods of bridging nerve gaps have been attempted and described during the past 70 years but none yet have been deemed to be uniformly satisfactory with respect to adequate end results

Following the conclusion of World War II a marked increase in the use of plastics developed and the rubber tubing used in ventriculo-cisternostomies was generally replaced with plastic tubing

Previous methods of nerve bridging described by Weiss[1 3 4] and others had not included the use of plastic tubing and it is the purpose of this paper

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to describe the results in several tubulization cases in which polythene polyvinyl and rubber were used in humans

These experiments were carried out in 3 human radial nerve injuries which were considered to be irreparable and which represented a small portion of the radial nerve injuries which had been treated during the past several years The series encompassed nerve deficits of from 7 to 15 cm in length or from 5 to 15 times the distance generally considered by Weiss[5] to retain a chance of union

METHOD

The first of these patients had a 12 cm deficit which was intubated over one year ago using rubber because polythene was not available at the time in sterile condition One centimeter of freshly divided proximal and distal nerve end was placed within each end of a segment of rubber tubing but was not sutured in place The subsequent patients had tubulization procedures with polythene and polyvinyl encompassing deficits of 10½ cm and 7 cm In 1 of these procedures the distal end was noted to slip out while waiting for a photographer and was subsequently fixed with a 0 003 tantalum suture through the tube wall and the epineurium of the proximal and distal segments thus formed a guide wire similar to that described by Matson[6] In each instance, 1 cm of the proximal and distal end was inserted into a snugly fitting tube segment one of which was highly flexible polyvinyl because of the fact that the deficit crossed the elbow joint and it was hoped to avoid the possibility of fibrous tissue getting through cracks in the tube wall There was no fluid interposed between ends at the time of operation in any case All of these patients began to show subjective recovery of pain and light touch sensation after surgery and each was believed to show return of sensation equal to that shown at relative times by other patients known to have almost complete return of function after primary repair All gave the appearance of a progressing Tinel sign Electromyographic studies were said to indicate simple motor units in the extensor carpi radialis and in the extensor hallucis longus in 1 patient after a 3 months interval from the time of surgery Function in these muscles was said to be present when the patient was examined in another hospital prior to removal of his tube In another patient denervation fibrillation was seen in the extensor digitorum communis on several occasions long after intubation

Three of the patients were able to read numbers written on the dorsum of the thumb Each of the major radial trunk injuries showed a decrease in the degree of wrist drop over a period of 6 months time and each had been continued on physiotherapy to the extensor muscle groups all during this time One of the 3 was able to hold his wrist in a position of optimum function identical to that demonstrated by a superficial radial transection at the junction site of sensory and motor trunk

Subsequently the 3 cases came to operation at intervals of from 7 weeks to 13 months Two of the 3 had been previously subjected to procaine median and ulnar blocks and had appeared to retain their sensory acuity in the major portion of the radial distribution Each had retained a small shield shaped area not over three fourths inch in diameter just proximal and slightly dorsal to the web of the thumb which was analgesic

The first tube case was subjected to operation at 9 months at which time

the skin broke down in the suture line at one end allowing an edge of the tube to protrude this episode being accompanied by micrococcus pyogenes drainage The tube was withdrawn and was completely unattached

This precipitated deliberate investigation of the case of 7 weeks duration at which time the proximal and distal centimeter of nerve were still found in almost identical positions in which they had been placed previously There was an interposed column of light tan gel made up of an almost solid mass of red cells with a wall of leukocytes and lymphocytes facing the proximal nerve end There was apparent viable nerve tissue by hematoxylin and silver stains extending to this wall of white cells and there was no evidence of any neuroma formation or regeneration of neurofibrils The sections of the distal end showed only a small amount of viable tissue but none beyond the original point of insertion of the distal nerve end

The last case was operated upon 13 months previously and was of considerable interest in that there was white fluffy tissue extending 1 cm beyond the proximal end and 1 cm through the polyvinyl tube from the distal end The gross appearance of these tissues was similar Again there was no evidence of a proximal or distal neuroma Interposed between the ends within the tube was a column of dark brown fluid which resembled the fluid seen in old subdural hematomas in contrast to another tube which contained a gel made up entirely of red cells but which had been examined much earlier Proximally there was indeed even at this late date a sharp line of demarcation demonstrated by silver stain however there was no wall of leucocytes as was seen at 7 weeks The proximal 1 cm extension showed no viable nerve cells and the distal end extension through the tube had only a few viable cells around its periphery which were believed to be Schwann cells

In the cases in which specimens of the surrounding tissues were obtained there were glistening membranes without the least bit of attachment to the rubber and plastic tubes as was noted by Spurling[7] while using tantalum These membranes seemed to originate adjacent to either end of the tubes and appeared to be closely adherent to the nerve trunk at these points

DISCUSSION

There was complete absence of regeneration in all these cases Clinically none of these patients were benefited by the procedure except for the striking absence of pain and scar tissue involvement and the apparent absence of neuroma formation This would seem to corroborate the opinion of W V Cone[8] relative to the use of plastic caps in neurectomies

Although these gaps far exceeded the experimental gaps tried in animals by Weiss[2,3,4] and Matson[6] it was of interest that overlap and trick motion seemed to progress with the rapidity of that seen in primary anastomosis with the ordinary direct approximation of nerve ends

The striking absence of regeneration might lead one to suspect that the tubing had just as deleterious an effect on the approaching neurofibrils as it did on the surrounding capillary tufts which on each occasion produced a laminated membrane with a smooth surface resembling a subdural membrane with an open space between the tube and membrane

Only the autonomous sensory area of a nerve can be considered in evaluating recovery and medicinal blocks of the median and ulnar nerve

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The first tube case was subjected to operation at 9 months at which time

ELECTROENCEPHALOGRAPHIC ALTERATIONS ASSOCIATED WITH EXPERIMENTAL ASYSTOLE AND VENTRICULAR FIBRILLATION IN DOGS*

SANFORD L. LEEDS, EDWARD SHEN AND HAROLD ROSENBLUM

It is well known that cardiac arrest may be followed by permanent cerebral changes if the heart action is not restored within a few minutes. Reversible alterations in the brain which follow severe cardiac arrhythmias such as standstill (asystole) or ventricular fibrillation can be demonstrated in the electroencephalogram. The electroencephalogram is being used routinely in the operating room during anesthesia in some institutions since it has been found to be a sensitive indicator of the condition of the patient. Many of the factors which alter the normal pattern of the electroencephalogram have been defined by clinical and laboratory studies. These include changes in the cerebral blood flow, hypotension, hypoxia, hypercapnea, etc.[5]

In the course of experiments in dogs on the etiology of ventricular fibrillation, continuous recordings of the electrocardiogram and electroencephalogram were obtained simultaneously while arrhythmias were produced. Cardiac standstill, ventricular fibrillation, bradycardia, ventricular tachycardia and other less severe arrhythmias were encountered. The purpose of this paper is to report the patterns reflected in the electroencephalogram of these cardiac arrhythmias as well as the time relations between the onset of the arrhythmias and the changes in the electroencephalogram.

METHOD

Mongrel dogs weighing about 12 kg. were used. They were in good physical condition and were free of cerebral pathology as far as could be determined.

Pentobarbital given intravenously was the anesthetic ("Veterinary Nembutal," Abbott, 29 mg./kg.). An endotracheal tube was inserted and either 100 per cent oxygen was administered by means of a Burn's valve or room air was administered by a mechanical apparatus which inflated and allowed deflation of the lungs. The heart was exposed through a left thoracotomy incision in the fifth intercostal space. The pericardium was opened routinely.

Ventricular fibrillation was produced by low voltage shock or by temporary coronary artery occlusion. In the latter group of dogs cardiac standstill (asystole) and other arrhythmias occurred, usually preceding the advent of ventricular fibrillation.

A 6 to 8 volt shock derived from a "Variac" was applied to the heart by means of electrodes. After ventricular fibrillation was allowed to continue for a variable length of time, defibrillation was carried out by a standard procedure: 100 per cent oxygen or room air was given under positive pressure, rhythmic cardiac compression was employed until the color of the heart approached normal, and then single or serial defibrillating shocks with voltages from 110 to 150 were applied with the electrodes in close contact to

* From the Harold Brunn Institute for Cardiovascular Research, Mount Zion Hospital, San Francisco 15, California. Supported by a grant from the United States Public Health Service.

The authors are grateful for assistance with these experiments rendered by Mrs. Eleanor Baird, Mrs. Doris Mitchell, Dr. Coleman Citret, Dr. Peter DeVries and Mr. Richard Wexler.

can be misleading just as they have been shown to be in surgical transections of these nerves for neuromata where the sequelae have been much more profound than they were after the previous procaine blocks

Numerous occasions of fairly discrete localization on stimulation of the distal segments have been noted in at least 10 per cent of our peripheral nerve cases and this was noted in the radial nerve transections occurring in the forearm This gross localization has been eliminated by procaine infiltration of the distal segment The reason for this phenomenon is not currently known but it appears to occur most frequently when the lesions are within a short distance of the hand

The Tinel sign which has led neurosurgeons to distraction on many occasions must have been transmitted through a jar on the snugly fitting tube s proximal end or the stimulus in some way passed thru collaterals in the hand In any event it is of doubtful value

There appears after injury to be a transition from the general zone of innervation to the autonomous zone over a period of months and certainly one cannot be certain about regeneration until this small autonomous zone shows signs of recovery

SUMMARY

1 Plastic and rubber tubing in lengths of 9 to 11 cm bridging gaps of 7 to 12 cm with no initially inserted intervening fluid have been uniformly unsuccessful with respect to nerve regeneration in humans

2 Patients have less pain and discomfort than comparable cases in which these tubes were not inserted and in which end to end suture was impossible

3 There does not appear to be any neuroma formation either proximally or distally in these tubes

4 The recalcitrance of the fibro-capillary tufts to approach these tubes may also be manifested by the regenerating axon

5 The possibility of management of neurectomies with plastic caps seems to be supported

6 The use of plastic tubing to bridge shorter gaps of 1 or 2 cm remains to be investigated

7 Direct approximation of nerve ends should be exploited by every possible means

8 Polyvinyl tubing remained in an elbow joint over a period of 13 months without cracking or allowing frank scar tissue to enter

REFERENCES

1 Weiss P Sutureless reunion of severed nerves with elastic cuffs of tantalum J Neurosurg *1* 219 225 1944

2 Weiss P Nerve regeneration in the rat following tubular splicing of severed nerves Arch Surg *46* 25 37 1943

3 Weiss P Nerve reunion with sleeves of frozen dried artery in rabbits cats and monkeys Proc Soc Exp Biol N Y *54* 274 277 1943

4 Weiss P and Taylor A C Histo chemical analysis of nerve reunion in the rat after tubular splicing Arch Surg *47* 419 447 1943

5 Weiss P and Taylor A C Nerve regeneration across gaps J Neurosurg *3* 375 389 1946

6 Matson D D Alexander E and Weiss P Experiments on the bridging of gaps in peripheral nerves of monkeys J Neurosurg *5* 230 248 1948

7 Spurling R G The use of tantalum wire and foil in the repair of peripheral nerves Surg Clin N America *23* 1491 1504 1943

8 White J C and Sweet W H Pain Its mechanisms and neurosurgical control 1st ed Springfield Ill Charles C Thomas 1955 p 422

The electrocardiogram was recorded simultaneously on the same paper as the electroencephalogram using 3 standard limb leads

RESULTS

Asystole or ventricular fibrillation was produced 79 times and was followed by resuscitative measures Sixteen dogs were used and the effects of the brain waves were observed in 14 In 11 of the 14 dogs which were studied with a continuous electroencephalographic record an electrocardiogram was simultaneously recorded

Although fibrillation and defibrillation can be carried out many times (11 times in dog number 382) for the most part this was repeated only 2 or 3 times in order that the animals might remain in as good a conditon as was possible

Low voltage shock (6 to 8 volts at 0 5 to 1 0 second) is followed consistently by ventricular fibrillation Defibrillation can always be accomplished if shocks of 110 to 150 volts at 1 to 5 sec are applied within a reasonable time (such as 1 or 2 minutes) if the animal s condition otherwise remains good As stated above recovery can follow the induction of as many as 11 or more experimental paroxysms of ventricular fibrillations

Ventricular fibrillation in 4 dogs (numbers 381, 382 383 384) was produced by means of low voltage shock alone In the remaining 12 a variety of arrhythmias including asystole and ventricular fibrillation were produced by means of temporary occlusion of part of the coronary arterial circulation In these animals low voltage shock was applied first in all but 1 dog (number 398) as a control followed by a defibrillating shock

The left anterior descending and circumflex coronary arteries were occluded by traction ligatures 26 times in 12 dogs An area of ischemia could be seen to develop over the left ventricle anteriorly and in the septal area extending to the right ventricle After the development of severe bradycardia cardiac standstill ventricular fibrillation or in one instance ventricular tachycardia the coronary arteries were released After release of the coronary arteries the ischemic area could be seen to regain a more normal appearance and the pattern of infarct or intense ischemia in the electrocardiogram usually improved or disappeared completely Although a number of arrhythmias were noted they could be divided into 2 main groups (1) ventricular fibrillation occurred in 15 instances after an average occlusion of 4 min 2 sec (extremes 1 min 30 sec to 15 min) (2) Cardiac standstill occurred 11 times after an average period of occlusion of 4 min 50 sec (extremes 2 min to 9 min 30 sec) Bradycardia preceded the standstill and usually lasted several minutes The cardiac rate gradually became slower and slower and finally the heart stopped Sometimes cardiac standstill was followed by ventricular fibrillation A short burst of paroxysmal ventricular tachycardia lasting 10 sec or less (Fig 3) often preceded the advent of ventricular fibrillation Other arrhythmias noted were A V dissociation 2 1 A V block isolated or grouped Wolff Parkinson White beats nodal ectopic beats and there was one instance of auricular fibrillation

The effects on the electroencephalogram of the phenomena outlined above may now be summarized Before doing this however it is necessary to describe the brain wave pattern in the anesthetized normal dog In the frontal leads there was a rate of 6 to 8 cycles per second with a 15 to 20

either side of the exposed heart When difficulty was encountered in defibrillation procaine was injected into the left ventricle in a few instances Epinephrine 1 1000 was used occasionally after defibrillation if the contractions of the heart were feeble

The second method for the production of ventricular fibrillation was temporary occlusion of part of the coronary circulation The left anterior descending coronary artery was isolated by dissection near its origin from the left coronary artery Occlusion of only the left anterior descending artery by traction on a ligature even when prolonged did not uniformly produce ventricular fibrillation Therefore the circumflex coronary artery also was isolated near its origin and a ligature passed under it By traction on the 2 ligatures one could observe an area of ischemia to develop followed by the development of arrhythmias which will be described

The electroencephalogram was recorded with a standard Grass III C 8 channel instrument The technique was standardized as follows after anesthesia was induced the dog s head was shaved between the supraorbital and occipital protuberances and between both ears The animals were grounded Recordings were made simultaneously from 4 areas of the scalp using as electrodes #26 gauge 3 inch needles which were connected to a jackbox The needles were placed so that odd numbers were on the left side and even numbers on the right side (see Fig 1) In this way leads 1 and 2 recorded frontally and leads 3 and 4 occipitally Before beginning each experiment an attempt was made to evaluate the brain wave pattern in each anesthetized dog

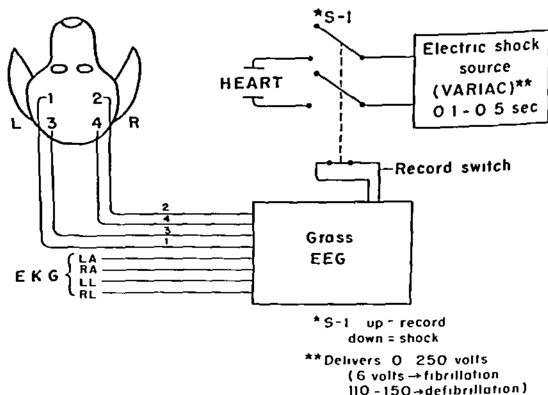


Fig 1 Illustrates circuit used in recording the electroencephalogram and electrocardiogram in the dog Source of shock (Variac) for producing ventricular fibrillation and defibrillation is shown

was no form remaining Eight to 10 seconds after the onset of ventricular fibrillation or asystole the brain waves completely disappeared in most of the dogs In 1 animal it was 20 seconds before the brain waves became flat All areas of the brain appeared to respond in this fashion simultaneously

After defibrillation of the heart and resumption of a normal sinus rhythm the form began to return within 7 or 8 seconds Usually there was a period of 1 to 2 minutes during which rhythmic cardiac compression was carried out and defibrillation shocks were applied During cardiac massage of course there is some blood circulating through the brain When a normal sinus rhythm is resumed the rate of the brain waves increased to 4 cycles per sec. After about 60 seconds a normal pattern was reestablished except for a slight decrease of potential (about 5 mv)

When bradycardia was produced and the pulse dropped in rate from normal to 60 or less a change in the brain wave pattern was noted The potential decreased and the form became more irregular and 6 seconds after the pulse fell to 30 the brain wave became flat When standstill occurred the pattern disappeared completely With a return of the heart beat to about 60 beats per minute or more the brain wave began to return and to resemble the normal pattern

As long as the pulse rate remained somewhere near normal the brain waves seemed to be fairly normal They might take on a slow rhythm and be disorganized but bursts of normal activity were also present

DISCUSSION

The relation of the cerebral blood flow and of hypoxia to alterations in the brain waves have been discussed at length in the literature[5 4] but the exact nature of the changes in the electroencephalogram are still poorly understood We have been unable to find any references to alterations in the electroencephalogram associated with experimental cardiac arrhythmias in dogs as reported here

Clowes *et al*[3] found the electroencephalogram to show cortical depression sooner with light or deep ether anesthesia when respiratory acidosis was present This report also indicated that the electroencephalogram was not particularly sensitive to anoxia during anesthesia in dogs for blood levels of 40 per cent or less oxygen saturation were necessary before changes in the electroencephalogram pattern were observed We have noted the same On the other hand Bellville *et al*[2 1] believe that the electroencephalogram is a more sensitive indicator of hypoxia depth of anesthesia and hypercapnea than are clinical signs They have published the electroencephalogram changes which occurred in a few patients with ventricular fibrillation They have also illustrated the rapidity with which alterations in the electroencephalogram appear when the great vessels are suddenly occluded by herniation of the heart through the pericardium

The relation of the various arrhythmias to the changes in the brain waves is of interest When severe bradycardia asystole or ventricular fibrillation occurred a rapid fall in the blood pressure was an obligatory concomitant The initial alterations in the brain waves occurred rapidly (in a matter of a very few seconds) and within 8 to 10 seconds the brain waves disappeared completely However if arrhythmias occurred without a concomitant disruption of the circulation sufficient to cause a fall in blood



Fig 2 Illustrates the normal electroencephalographic patterns in the dog and the simultaneous electrocardiogram

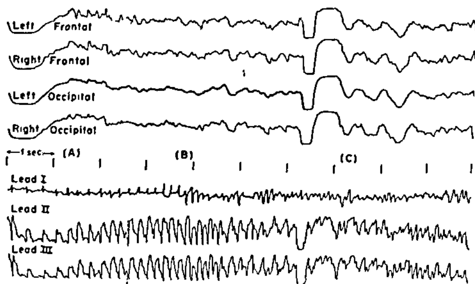


Fig 3 Illustrates the change of the brain waves from a normal (A) to a deteriorating slow flat pattern (B) and (C) The electrocardiographic pattern of normal rhythm (rate 156) changes to (B) ventricular tachycardia (rate 330) followed by (C) ventricular fibrillation

microvolt potential It was a regular synchronous sine wave pattern The occipital leads showed a 10 to 20 microvolt potential and a 4 to 5 cycle per second synchronous sine wave (Fig 2)

With asystole or ventricular fibrillation the electroencephalographic changes could be noted in 4 to 6 seconds in 19 experiments (Fig 3) The rate dropped to 2 to 3 cycles per sec from the usual 6 to 8 cycle frontal and 4 to 5 cycle occipital rates The potential rose to about 50 microvolts and then dropped gradually to zero The form became irregular with loss of the sine wave pattern and then assumed bizarre characteristics until there

Orthopedic Surgery

INTRODUCTION

RALPH K. GHORMLEY

In assuming the position of moderator as a replacement for the late J. Albert Key, I am well aware of the fact that no one can replace him in our meetings. His constant interest in all phases of medicine, his appreciation of the importance of research in medicine and his effort to help in the development of this program were known to all. All of us sustained a great loss in his death.

The subjects presented in this Orthopedic Section of the Forum Volume may be grouped under bone grafting, bone replacement, bone growth, bone sterilization and bone healing. Dr. Hartley's experiments show beyond a doubt that bone grafts attached to a muscle pedicle will live, whereas free grafts die. Dr. Bonfiglio's experiments clearly demonstrate the greater viability of fresh autogenous grafts over that of fresh homogenous grafts and the much greater tendency of such grafts to heal after fracturing than is true of those grafts prepared in the various ways to which bone bank grafts are subjected.

Dr. Bliven's study of the uptake of P^{32} in ununited fractures is interesting because it demonstrates the greater uptake of this material in fracture callus as opposed to cortical bone, leading to the conclusion that vascularity and viability of bone are not the causes of delayed union in his study.

Ray, Wolff, Thomson and LaViolette, using strontium^{90}, studied the uptake of inorganic salts in living and dead bone and also the mobilization of these salts from similar areas of bone. Their conclusions that the uptake of inorganic salts is not dependent upon viability of bone and that more rapid mobilization of these salts takes place from living than from dead bone present an interesting and important viewpoint. It helps to explain the increased density of bone in areas of aseptic necrosis.

Greville and Janes, in a carefully controlled group of experiments, demonstrated that fractures in living young animals heal with a pretty constant stimulus to increased growth of the bone fractured. Liebolt studied the effect of the introduction of intramedullary pins in the bones of young dogs. He found that the growth of the bone, both in length and diameter, is altered although ultimate length is obtained when the epiphyseal line has not been damaged.

Two papers are presented on the sterilization of bone grafts by radiation. DeVries, Kempe and Brinker sterilized grafts in experimental animals by means of irradiation from radioactive cobalt. They have demonstrated that effective sterilization can be accomplished without apparent deleterious effect on the grafts. Sterilization of banked bone for use in human beings

pressure the electroencephalogram pattern remained good This was particularly true if the pulse rate remained somewhere near the normal level in the anesthetized dog When the heart returned to a normal rhythm spontaneously or after resuscitation the electroencephalogram rapidly approached the normal within 7 or 8 seconds

Libet and Gerard[6] and others have stated that the electrical potential recorded by the electroencephalogram is dependent on the available energy supplied by the metabolism of the neurones The rapidity of the changes noted in the electroencephalogram when the circulation is cut off by cardiac arrest or torsion of the great vessels due to herniation of the heart make the relationship to the blood flow quite apparent

SUMMARY

1 Asystole ventricular fibrillation, bradycardia ventricular tachycardia and other arrhythmias were produced in dogs by means of (a) low voltage shock and (b) temporary occlusion of a portion of the coronary arterial circulation

2 Simultaneous electroencephalographic and electrocardiographic records were obtained which showed that electroencephalographic changes occur within 1 to 6 seconds after cardiac arrest and the brain waves usually disappeared after 8 to 10 seconds These changes were reversible and a fairly normal brain wave was reestablished 8 seconds after the heart was returned to a normal sinus rhythm

3 The minor arrhythmias which are not associated with marked disturbances of the circulation did not affect the electroencephalogram remarkably

4 Abnormalities in the electroencephalogram occurred regularly and almost immediately with those disturbances of the heart beat known to be incapable of producing an effective blood flow

REFERENCES

1 Bellville J W Artusio J F Jr and Glenn F The electroencephalogram during cardiac manipulation Surgery *38* 259 1955
2 Bellville J W Artusio J F Jr and Glenn F The electroencephalogram in cardiac arrest J Am M Ass *157* 508 1955
3 Clowes G H Kretchmer H E McBurney R W and Simeone F A The electroencephalogram in the evaluation of the effects of anesthetic agents and carbon dioxide accumulation during surgery Ann Surg *138* 558 1953
4 Corday E Rothenberg S F and Putnam T J Cerebral vascular insufficiency A M A Arch Neur Psychiat *69* 551 1953
5 Hill D and Parr J Electroencephalography New York The Macmillan Company 1950
6 Libet B and Gerard R W Control of the potential rhythm of the isolated frog brain J Neurophysiol *2* 153 1939

REPAIR OF BONE TRANSPLANT FRACTURES*

MICHAEL BONFIGLIO

The introduction of the bone bank in the last few years has been accompanied by a revival of the old controversy as to which type of bone transplant is superior. The majority of investigators conclude that fresh autogenous bone is superior to other types of transplants such as fresh homogenous bone, frozen homogenous bone, freeze dried, and merthiolate stored homogenous bone. However, many investigators believe the difference is not sufficiently significant to warrant the additional risk to the patient or additional expenditure of time by the surgeon to obtain the transplant. The advocates of this latter idea support the use of bank bone either frozen, freeze-dried or merthiolate preserved. Recent experimental and clinical studies by Wilson,[1] Reynolds and Oliver and Kreuz *et al*[3] have led to the opinion that the healing of banked bone, although somewhat slower to repair in the earlier stages than fresh autogenous bone, was eventually as good or better than fresh autogenous bone. These investigators maintain that all transplanted bone dies and host connective tissue alone is responsible for invasion and replacement of the graft as a scaffold.

In an attempt to evaluate the problem, experiments were undertaken in rabbits to study the repair of fractures in bone transplants of various types. Fresh, frozen and boiled autogenous transplants and fresh, frozen, freeze dried and merthiolate preserved homogenous transplants were studied. Graft fracture healing was used as a criterion of the repair capacity of the transplant since host bone callus would be less likely to influence that site. Graft fracture healing would then depend on the type of transplant and the host connective tissue response to the graft.

METHOD

Large young male adult rabbits were used. Three centimeter segments of bone with periosteum were removed from the right ulna, fractured at midpoint and replaced in the same defect for autogenous fresh grafts. Fresh homogenous transplants were accomplished by direct transfer of ulnar segments from pairs of rabbits. Frozen grafts were stored in sterile double glass containers at −15 to −20 C for periods of 1 to 12 weeks before use. Freeze dried *grafts were sealed in glass tubes under vacuum and stored* at room temperature. At the time of operation the freeze dried grafts were placed in normal saline before they were fractured and placed in the rabbit. Bone segments were stored in 1:1000 merthiolate solution at least 1 week before use. The animals were sacrificed at intervals from 1 to 12 weeks after insertion of the fractured grafts. One hundred rabbits are included in the final study. Celloidin sections were prepared for histologic study.

RESULTS

Figure 1 summarizes the results of graft fracture healing.

Autogenous Transplants. In 10 of 15 rabbits fresh autograft fractures

*From the Department of Orthopedic Surgery, State University of Iowa, College of Medicine, Iowa City, Iowa. This work was aided in part by grants from the Douglas Smith Foundation, University of Chicago, The National Institutes of Health, United States Public Health Service, and the Central Scientific Fund, College of Medicine, State University of Iowa.

by means of cathode rays is reported by Bassett Hudgins, Trump and Wright That this produced effective sterilization seems well demonstrated by the authors although the ultimate effect of this treatment on the graft seemed to remain in doubt

Bovill showed that while hypervitaminosis A slows epiphyseal growth temporarily ultimate growth is not altered

Peltier and Lillo s experiments in the replacement of segments of bone in experimental animals by sterilized plaster of Paris rods show that the plaster of Paris ultimately is completely replaced by bone and that while the plaster is being replaced the blood calcium of the animals is elevated

Smith and Dunsford produced fractures in bones rendered osteoporotic by sectioning the sciatic nerve of rats they found that healing of these fractures is much less firm and complete than in control animals without osteoporosis

While the practical application of some of these experiments to orthopedic surgery seems far off each of them has contributed to some phase of the physiology of bone in a manner that may be useful in developing further knowledge of this important subject

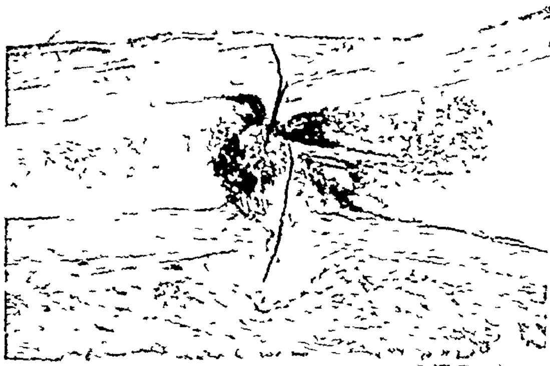


Fig 3 Two week fresh homogenous bone transplant fracture with non union and cellular reaction

bony union of the transplant fracture none of which occurred in less than 5 weeks In the first few weeks the fracture site showed invasion of host granulation tissue with marked round cell infiltration (Fig 3) The granulation tissue changed to fibrocartilage cartilage and bone in those instances where union occurred At the end of 12 weeks the animals with non union demonstrated mature less cellular fibrous tissue at the fracture site The homograft bone replacement was first by vascular connective tissue invasion bone absorption and later ossification of the fibrous tissue Direct creeping substitution as usually seen in autografts was less evident In addition a round cell and eosinophil inflammatory reaction occurred between the graft and overlying muscle This was most intense at 3 weeks and gradually subsided but did not completely disappear as long as unreplaced bone remained This appears to be an immunological response (Bonfiglio Jeter and Smith)[4]

Frozen Homogenous Transplants Bony union resulted in only 1 (a 6 week old animal) of 22 rabbits The others united by fibrous tissue only (Fig 4) The usual type of fracture repair was conspicuous by its absence Frozen homografts displayed a lack of osteogenic stimulus to the mesenchymal cells of the surrounding connective tissue The absorptive reaction present in fresh homografts was less evident particularly in the early weeks At the end of 12 weeks less than 20 per cent of the transplant had been replaced by either fibrous tissue or bone Cellular inflammatory reaction to the frozen homograft was less intense than in fresh homografts

Freeze-dried Homogenous Transplants Fibrous non union occurred in all of the animals No attempt at fibrocartilage cartilage or bone formation at the fracture site could be noted Cortical repair was similar to that observed for frozen homografts The cellular inflammatory reaction was minimal

united solidly with bone as early as 2 weeks The appearance of the fracture repair (Fig 2) is similar to that of an ordinary fracture in a rabbit Three of the graft fractures had a cartilaginous union and 2 had a fibrous union As early as 1 week periosteal new bone apparently arising from proliferating periosteal or surface bone cells was observed Within 12 weeks almost complete replacement of the transplant by living bone had taken place

Fresh Homogenous Transplants Seven of 23 animals in this group had

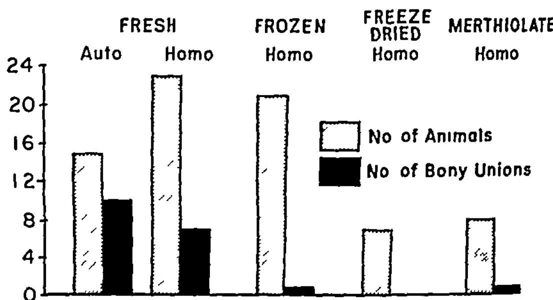


Fig 1 Repair of bone transplant fractures

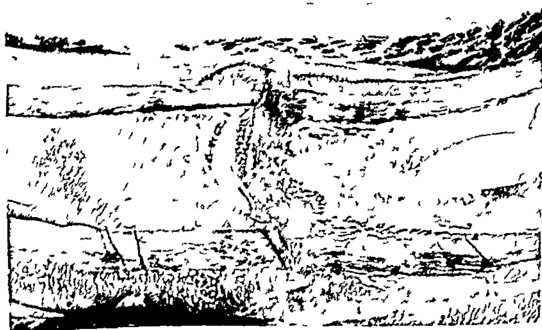


Fig 2 Two week fresh autogenous bone transplant fracture with bony union

REFERENCES

1 Wilson P D Experiences with a bone bank Ann Surg 126 932 946 1947
2 Kreuz F P Hyatt G W Turner T C Bassett C A L The preservation and clinical use of freeze dried bone J Bone Surg Brit Vol 33 A 863 872 1951
3 Reynolds F C Oliver D R Ramsey R Clinical evaluation of the merthiolate bone bank and homogenous bone grafts J Bone Surg Brit Vol 33 A 873 883 1951
4 Bonfiglio M Jeter W S and Smith C L The immune concept Its relation to bone transplantation Ann N York Acad Sc 59 417 433 1955

P^{32} EXCHANGE IN DELAYED UNION OF FRACTURES*

FLOYD E BLIVEN JR AND JEAN O BOYD

The uptake of radioactive phosphorus has been applied experimentally and clinically to bone viability In fracture of the femoral neck low specific activity of P^{32} in the head as compared with the specific activity of the trochanter or distal neck has been cited as indicating impaired blood supply of the head or aseptic necrosis of the head In animals the increased P^{32} uptake in a fractured bone has been explained on the basis of increased vascularity and increased local metabolism The technique was applied in this study to the problem of delayed healing of fractures in which similar pathological factors may exist

Delayed healing or delayed union is considered to be a local defect in vascularity Scar tissue disorganized cartilage or fibrinoid degeneration have been described replacing the osteogenic process of callus organization Yet if a fracture is adequately immobilized it almost invariably heals even though years may be required Many surgical procedures will accelerate osteogenesis It was hoped that P^{32} uptake might guide the surgeon in the indication and timing for such intervention

Two patients with ununited fractures were studied On the day before surgery for nonunion radioactive phosphorus was given orally At operation samples of bone were taken from the fracture callus at the site of delayed healing from adjacent bone and from non fractured bone In addition to determinations of the radioactive phosphorus activity of each sample the bone was analyzed for its mineral and water content and histological sections were obtained

The first patient was a 55 year old male with a shrapnel wound of the leg involving a large tibial shaft and soft tissue defect Several reconstructive procedures were done to bridge the defect these included transplantation of the fibular shaft to the proximal and distal tibial fragments an onlay cortical graft from the opposite tibia spanning the defect and autogenous iliac bone applied posteriorly to the graft The distal ends of the tibial graft and the fibular transplant fused However proximal fusion failed to occur and after 2 years the leg was amputated The patient received 0 614 mc of P^{32} by mouth and samples were obtained from several sites as illustrated in Figure 1 Motion was easily demonstrated at the

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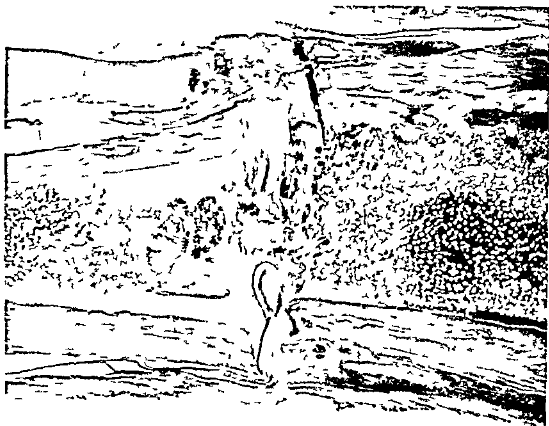


Fig 4 Fibrous union of 7 week frozen homogenous bone transplant fracture Cortical replacement by tissue without new bone formation

Merthiolate Homogenous Transplants In 2 of the rabbits partial bony and cartilaginous union occurred Otherwise the bone repair and reaction are similar to those of freeze dried and frozen homografts Autogenous boiled and frozen transplants show very poor graft fracture repair capacity

SUMMARY AND CONCLUSIONS

Fresh autogenous bone transplant fractures have the capacity to unite within 2 weeks Some of the transplant surface cells appear to survive and proliferate to produce new bone The graft replacement is by creeping substitution The transplants are well tolerated by host tissues

Graft fracture healing in fresh homogenous transplants is delayed several weeks and less than one third of the fractures united The grafts replace by fibrous tissue first before ossification occurs Fresh homogenous transplants induce a cellular inflammatory reaction similar to that described for other homograft tissue Frozen freeze dried and merthiolate homogenous bone transplants produce little osteogenic stimulus in the host tissues Graft fractures usually do not unite These grafts replace very slowly if at all by fibrous tissue first and then bone

Fresh autogenous bone transplants are far superior to any other type of bone transplant The repair capacity of fresh autografts as measured by graft fracture healing exceeds that of fresh frozen freeze dried or merthiolate homografts

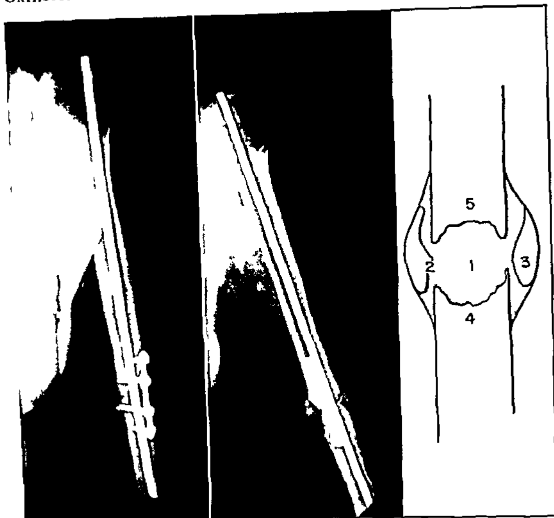


Fig 2 Patient D U Lateral and A P films of humerus Location of bone samples as illustrated Two cortical samples were taken with a graft from the opposite tibia

active new bone formation (Fig 3) Throughout the sections examined were areas of hyaline cartilage fibrocartilage, fibroblasts and calcifying new bone In the adjacent areas granulation tissue and fibrous elements predominated The cortical sections were composed of thick lamellar trabeculae and there was little evidence of cellular activity except for the presence of the osteocytes in the lacunae The grafts contained many empty lacunae The osteoporotic bone was characterized by thin trabeculae large Haversian spaces and no new bone

The mineral content was remarkably uniform in all samples The chemical differences were in the proportions of water and ash The callus showed more water and less ash than cortical bone while osteoporotic bone held less water

The specific activity of P^{3} was high in the samples of callus and osteoporotic bone and low in cortical bone In the younger patient this activity in callus reached 50 times the concentration of P^{3} in cortical bone though the mineral content was one third less Related to the mineral content the P^{3} uptake in callus was therefore 150 times the uptake in cortical bone

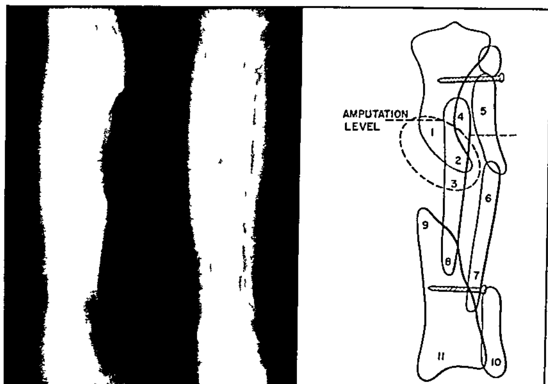


Fig 1 Patient W T Lateral and A P films of leg Diagram shows location of bone samples The fracture is bridged by a tibial onlay graft and the transplanted fibula Dotted circle indicates callus at non union site

proximal junction of the tibial graft and the proximal tibial fragment this junction was included in a firm white callus The proximal junction of the fibular transplant and the tibia allowed slight motion when stress was applied Thus in the amputated extremity 3 degrees of healing were demonstrated non union allowing motion partial or delayed union allowing motion only with stress and firm union Samples were also taken from distal tibia and fibula which were osteoporotic as shown in the x ray

The second patient was a 25 year old female in excellent health who had incurred a fracture of the midshaft of the humerus with other injuries in a fall The fracture was originally treated by open reduction and fixation with a plate and an intramedullary nail after fixation autogenous grafts of iliac bone were applied around the fracture When the humerus failed to unite after 1 year the metal was removed and an onlay tibial graft was applied Prior to this surgery 0 096 mc of radioactive phosphorus were given Bone samples were obtained from the medullary callus the peripheral callus incorporating the iliac grafts the cortical bone adjacent to the callus and the tibial donor area (Fig 2)

The results are presented in Tables 1 and 2 and are similar in the 2 patients The data point out differences in callus as compared to cortical bone and also differences which identify osteoporotic bone There were no significant differences in samples from different bones or in the comparison of fractured bone graft and bone transplant with the exception that bone near the fracture site had some of the findings associated with callus

The callus in both patients despite the long period since injury showed

Table 2 Patient D U

Specimen Number	Location of Specimen	Composition: Phosphorus Mg Phosphate per Gm Ashed Bone	Composition: Water per Cent Wet Weight	Composition: Ash per Cent Wet Weight	Composition: Counts per Min per Mg Phosphorus	P^{32} Activity: Percentage Dose P^{32} per Gm Phosphorus	P^{32} Activity: Percentage Dose P^{32} per Gm Bone Wet Weight	Histology
	Callus at site of delayed union							
1	*Center* of callus	533	66.2	15 0	20 98	0.271	0 022	Active new bone formation cartilage and fibroblasts
2	Margin of callus and autogenous *iliac graft*	551	45.2	28.5	10.27	252	039	
	Cortical bone near fracture							
3	Autogenous iliac graft at fracture site	536	19 0	59 1	3 45	015	012	Thick trabeculae surrounded by granulation tissue In some areas there are osteoblasts and new calcification
4	Humerus shaft just distal to callus	512	47 4	27 0	8.36	109	015	
5	Humerus *shaft* just proximal to callus	560	24.5	48 0	4 11	054	015	
	Cortical bone from non fractured bone							
6	Tibia cortex donor graft proximal	547	15 1	44 0	0.352	005	001	Thick lamellar bone
7	Tibia cortex, donor graft distal	510	13 8	48 0	0 326	004	001	

Table 1 Patient W T

SPECIMEN NUMBER	LOCATION OF SPECIMEN	COMPOSITION					P^{32} ACTIVITY		HISTOLOGY
		CALCIUM MG CALCIUM PER GM ASHED BONE	PHOSPHORUS MG PHOSPHATE PER GM ASHED BONE	WATER PER CENT WET WEIGHT	ASH PER CENT WET WEIGHT	COUNTS PER MIN PER MG PHOSPHORUS	PERCENTAGE DOSE P^{32} PER GM PHOSPHORUS	PERCENTAGE DOSE P^{32} PER GM BONE WET WEIGHT	
	CALLUS AND NON UNION SITE (DEFINITE MOTION)								
1	Tibia proximal	390	542	17 8	49 0	4 52	0 016	0 012	Many osteogenic elements hyalin and fibrocartilage cellular fibrous tissue
2	Tibia proximal	404	574	21 5	45 5	3 89	010	010	
3	Tibial graft middle	396	577	28 3	43 0	5 14	036	014	
	DELAYED UNION SITE (MODERATE MOTION WITH STRESS)								
4	Tibial graft proximal	378	548	15 8	51 0	1 93	020	006	Areas of new bone with columns of osteoblasts alternating with areas of organized fibrous tissue
5	Transplanted fibula	393	541	13 6	34 0	2 24	023	001	
	CORTICAL BONE								
6	Transplanted fibula middle	391	558	11 7	45 0	1 00	010	003	Thick trabeculae with osteoblasts and small areas of fibrous tissue
7	Transplanted fibula distal at site of union	381	529	12 5	50 0	1 20	012	003	
8	Tibial graft distal at site of union	389	590	20 8	46 0	1 32	013	001	
9	Tibia middle sclerotic spur	396	520	20 8	39 0	1 53	016	003	
	DISTAL OSTEOPOROTIC BONE								
10	Fibula distal epiphysis	411	586	8 6		3 89	010		Thin trabeculae
11	Tibia distal epiphysis	410	629	19 5		8 14	083		No new bone Dilated Haversian canals

site of osteogenic activity The large numbers of cells suggest adequate circulation The transport of inorganic ions across the fracture area is rapid If crystal surface is a factor in new crystal formation the callus presents many times more the available surface of established bone and would seem an ideal situation for further ossification

Osteoporotic bone also offers a larger adsorption surface This appears to be effected by invasion of cortical bone by larger Haversian vessels exposing more bone and by narrowing of the trabeculae enhancing diffusion

This study does not establish a cause for delayed healing It does demonstrate in the unhealed bone mineral exchange cellular nutrition and osteogenic elements which suggest that with further stimulation the callus might become solid bone One might speculate from these findings that the cause of defective healing is deficient production or organization of the organic matrix The apatite crystals are known to be oriented along the collagen fibers As bone becomes established these fibers become aligned forming masses of tight matrix and packed crystals Cortical bone is the culmination of this process of orientation rigidity and reduced adsorption surface and is disturbed only by the erosive or invasive processes of osteoporosis

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STUDIES OF BONE GROWTH FOLLOWING EXPERIMENTAL FRACTURES OF THE FEMUR IN PUPPIES*

NICHOLAS R GREVILLE AND JOSEPH M JANES

One of the factors known to influence growth of bone locally is fracture of the growing bone This is of practical importance in treating fractures in children especially fractures of the femur it is however of theoretical interest as well in that it gives a fairly simple method of studying the ability of local influences to alter growth locally In this experiment the effect on growth of different fractures of the midshaft of the femur in puppies was studied

METHOD

Twenty five puppies 3 to 4 months of age were used They were divided into a control group and 4 experimental groups with 5 puppies in each group

Each puppy in the control group had 3 small stainless steel screws inserted in 1 femur 1 screw was placed in the lower portion of the epiphysis and 2 were placed about 1 cm apart at the center of the diaphysis Each screw had a hole punched in its head to ensure that the measurements between each one taken by calipers were accurate at all times The other femur was not disturbed

The 4 experimental groups were numbered I to IV inclusive

Group I In this group the screws were placed as in the control group and the femur was divided transversely between the diaphyseal pair of

*From the Department of Orthopedic Surgery The Mayo Clinic and Mayo Foundation Rochester Minnesota



Fig 3 a (Patient D U Specimen 1) 100 X Fracture callus Showing new bone formation from fibrous osteoid tissue and cartilage

b (Patient D U Specimen 2) 100 X Apposition of autogenous iliac graft and fracture callus Graft is on left new bone middle fibrous callus right

c (Patient W T Specimen 3) 100 X Non union site Tibial graft invaded and surrounded by thin margin of osteogenesis and fibrous tissue and scar

d (Patient W T Specimen 8) 100 X Area of fusion Graft on right and tibial fragment left joined by bridge of new bone and united in fibrous tissue

e (Patient D U Specimen 1) 100 X Cortical Bone Solid lamellar trabeculae with lacunae and small Haversian vessels

f (Patient W T Specimen 11) 100 X Osteoporotic bone Thinned trabeculae and dilated with Haversian vessels large spaces filled by marrow and fat

In the interval of this investigation the specific activity or uptake of radioactive phosphorus is primarily a surface exchange reaction It is dependent upon adsorption on the available exchange surface of the apatite crystals Exchange begins quickly after administration Many studies have shown that at 24 hours the equilibrium is almost entirely limited to the inorganic phosphate exchange between the blood and bone Exchange is greater in areas of bone where the number of the crystals is less the hydration greater and the crystals more recently formed Callus is such a region This exchange required only a diffusion of the P^{32} through callus and bone and does not necessarily register increase or decrease in blood supply

From these data the callus is illustrated even months after injury as a

site of osteogenic activity The large numbers of cells suggest adequate circulation The transport of inorganic ions across the fracture area is rapid If crystal surface is a factor in new crystal formation the callus presents many times more the available surface of established bone and would seem an ideal situation for further ossification

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Nicholas R. Greville and Joseph M Janes

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* From the Department of Orthopedic Surgery The Mayo Clinic and Mayo Foundation Rochester Minnesota



Fig 1 (a) Left Transverse fracture viewed after operation (Group I) note that fracture is fixed by pin with bones in end to end apposition (b) Right Same fracture as shown in Figure 1a after healing full growth has taken place

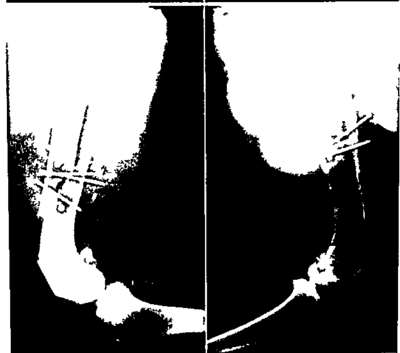


Fig 2 (a) Left Oblique fracture in Group III viewed after operation fracture is fixed with overriding of fragments held in place by multiple wires (b) Right Same fracture as shown in Figure 2a after healing full growth has occurred

screws by means of 3 drill holes through both cortices dividing the bridges between the holes with an osteotome The fragments were held in end to end apposition by an intramedullary pin (Fig 1a) The distances between the diaphyseal screws before fracture and after fixation and the distance between the lower diaphyseal screw and the epiphyseal screw were measured with calipers

Group II The 5 pups in this group were treated similarly except that their femora were divided obliquely

Group III The femora were again divided obliquely between the diaphyseal screws but the fragments were allowed to override and were pinned

in the position which they naturally assumed with 3 short pieces of Kirschner wire (Fig 2a) In 1 pup 2 screws were used and in 1 other the fragments could not be fixed The amount of overriding was measured

Group IV The femur was divided transversely, the bone ends were displaced and allowed to shorten and the fracture was stabilized in the same way as in Group III One femur was fixed with screws

In 1 puppies the nutrient artery to the femur was identified and divided

Follow up Study Monthly roentgenograms were taken of each puppy until union of the fracture had occurred In 3 pups the pin was removed after union and the screws were removed from both the pups in which they were used

All the puppies survived all the fractures united and in no instance were there any complications

When the pups were estimated to be fully grown, at between 10 to 12 months of age roentgenograms of the fractured femora were made to see if the epiphyses had closed (Figs 1*b* and 2*b*) When this had occurred the animals were anesthetized and killed The bones of both hind limbs were removed and boiled together until clean of all soft tissue they were then dried and their lengths estimated by a modified anthropometric board The distances between the measuring screws were measured by calipers

Thus the following data were obtained for each puppy (1) difference in length of fractured and undisturbed femora (2) growth of fractured femora owing to lower femoral epiphysis and (3) shortening and absorption at site of fracture

The increase of growth on the fractured side was found by substracting the difference in length between the fractured and the untouched femora—the ultimate shortening—from the sum of the shortening measured at operation and the subsequent absorption or slip at the fracture site—the total shortening

RESULTS

The average increase of growth for each group has been *correlated on* the composite graph (Fig 3) with the total shortening at the fracture site the difference in lengths of the fractured and intact sides and the total growth due to the lower femoral epiphysis during *the period of* observation also are shown The graphs show that these 4 factors increase from group to group Group I showing the smallest and Group IV the largest figures for all 4 measurements

The control group with screws but without fracture showed an insignificant increase of growth over the untouched side an insignificant increase in length 2 cm of total growth and no shortening

Group I with transverse fractures fixed in end to end apposition showed an average increase in growth of 0.24 cm, 0.3 cm absorption at the fracture site slight ultimate shortening of the fractured femur and 1.7 cm total growth

Group II with oblique fractures fixed with end to end apposition showed an increase of growth of 0.5 cm 0.7 cm absorption 0.2 cm ultimate shortening and 2.3 cm total growth

Group III with oblique fractures fixed with overriding showed an increase in growth of 1.0 cm with 1.0 cm overriding and 0.6 cm absorp

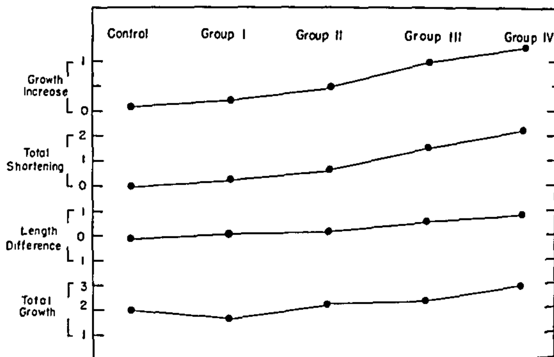


Fig 3 Composite graph showing effect of fracture and displacement in each group on factors shown on left side

tion or slip making 1.6 cm total shortening at the fracture site, ultimate shortening of 0.6 cm and total growth of 2.1 cm

Group IV with transverse fractures fixed with overriding showed an increase in growth of 1.3 cm with 1.9 cm overriding and 0.4 cm absorption or slip at the fracture site making a total shortening of 2.3 cm, ultimate shortening of 0.9 cm and total growth of 3.0 cm

Ligation of the nutrient artery, variation of the type of fixation or removal of it did not seem to affect the results

COMMENT

From these results it may be seen that an increase of growth followed all the fractures. This increase was not in any case sufficient to make up completely for the shortening that occurred but in Groups III and IV it approached half the total growth, the average amount by which the lower epiphysis would have increased the length of the bone had it not been fractured was almost doubled by the procedure in these groups. The increase was greatest with the greatest amount of displacement and in the undisplaced fractures it was greater in the oblique fractures (Group II). Callus formation was also greatest in the fractures with most displacement

Insertion of screws into the diaphysis and epiphysis did not produce significant increase of growth, neither did ligation of the nutrient artery

CONCLUSIONS

From our study of fractures of the midshaft of the femur in 25 puppies we have concluded that growth of a bone is increased by fracture of the shaft of the bone while it is growing. The increase depends on the amount of displacement and the type of fracture and takes place at the lower part of the epiphysis. The increase may equal the expected normal growth

Practically if a child suffers a midshaft femoral fracture the length as well as the alignment and rotation of the bone should be restored in those patients in whom the fracture has not resulted in much displacement or comminution If the fracture has resulted in severe displacement full length should not be restored though some length should be

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THE EFFECTS OF INTRAMEDULLARY METALLIC NAILS ON GROWING BONE*

A Preliminary Report

Frederick Lee Liebolt and Edward H Wilson Jr

It is known that intramedullary nails in adult bone produce no untoward changes under normal conditions Because of this there has been a trend to use the same type of metallic nails in children particularly in the treatment of fractures However it is worthy to consider that intra medullary nails in growing bone might produce atrophy or hypertrophy elongation or shortening or aseptic necrosis In view of these unknown factors the present experiment has been undertaken to determine the effects of different types of nails on growing bone when the nail is placed only in the shaft of the bone and not across the epiphyseal growth lines and when the nail is placed in the shaft of the bone and across the epi physeal growth lines

The plan of the experiment was to obtain pups of a known age to use dogs of the same litter to await growth until sufficient age for surgery to operate upon all the animals under identical conditions using the same technique and the same instruments to use the right radius for the im plantation of the nail and the left radius as the growth control to insert the 3 types of orthopedic nails in common usage the round or Rush the U shaped or Kuentscher and the triangular or Street to place the nails in the first series only in the intramedullary canal and not across the epiphyseal lines and in the second series to place the nail not only in the intramedullary canal but also across the epiphyseal lines and in the third and fourth series to remove the nail before closure of the wound as the operative control to take x rays of both front legs every 4 weeks until the pups were fully grown for comparison of the bone growth and to study by pathological section the bones of those animals which died during the interim

Pups of a known age were obtained by transferring pregnant dogs to the animal quarters of the Cornell University Medical College Following birth and adequate development in size and strength 23 were subjected to 6 surgical procedures under intravenous sodium pentothal anesthesia X rays were taken of both front legs for a record for future measurements and for the size and length of the medullary cavity of each radius The

*From the Department of Surgery (Orthopedics) of the New York Hospital Cornell Medical Center New York N Y This study was aided by a grant from the Marie Heye Clemens Fund Inc

right leg was shaved and under sterile conditions a 2 cm incision was made on the lateral aspect of the distal extremity of the right radius and in one series at the proximal extremity also A drill hole was made from the surface of the bone to the medullary cavity Each medullary nail was 2 mm in diameter was approximately the same size as the medullary canal, and was sufficiently tight that it could not be inserted by the fingers but could be inserted by the use of a pair of pliers Under x ray control the nail was pushed up the medullary canal between the epiphyses and in one series across the epiphyses The periosteum was closed over the drill hole by the use of interrupted 1 0 plain catgut sutures The sub cutaneous tissues were united in a like manner and the skin edges were approximated by interrupted vertical mattress cotton sutures A dry dress ing was applied by collodion Final x rays anteroposterior and lateral views of the right radius were taken and the puppy was returned to his cage One week later the puppies were walking without a limp The dressings remained *in situ* along with the sutures, and there was no evidence whatsoever of infection The puppies were sent to the country to be boarded on a farm until they became fully grown which was estimated to be one year

The first series of experiments was undertaken on 5 puppies at the age of 10 weeks A round Rush nail was inserted into the shaft of the right radius between the epiphyses One puppy accidentally died 8 months after operation and the 2 radii removed at autopsy revealed the radius which contained the round Rush nail to be shorter and thicker in com parison with the control radius although the proximal and distal epiphy seal lines were relatively equal in width in both bones However, measure ments of the x rays of the remaining 4 animals, taken at full bone maturity with the epiphyseal lines closed revealed no differences in the length and the width of the radii

The second series of experiments was undertaken on a litter of 6 puppies at the age of 8 weeks In this group the technique was exactly the same except that the type of nail placed in the medullary canal of the right radius was a 2 mm U shaped Kuentscher nail One of the puppies died 2 months after the operation and the 2 radii removed at autopsy again showed that in only 2 months time the radius containing the nail had produced a shortening and a broadening of the bone in comparison to the control radius However x ray measurements of the 5 dogs at full bone maturity again revealed no differences in the length and the width of the operated and unoperated bones

Apparently the implantation of intramedullary nails in the shaft of the radius produces a temporary derangement in the development of the bone which however disappears by the time full bone maturity is reached

The third series of experiments was started on a group of 6 puppies at the age of 2 months but had to be abandoned Originally it was intended to use a triangular Street nail but these could not be purchased smaller than 3 mm in diameter and upon forcing the pin into the medullary canal the cortex of the bone fractured The triangular type of nail therefore was discarded and the experiment discontinued after being attempted on only one puppy

The fourth series of experiments was performed on one litter of 6

puppies aged 8 weeks, by placing a 2 mm U shaped Kuentscher nail not only in the medullary canal of each right radius but also across the proximal and distal epiphyseal lines into the proximal and distal epiphyses This procedure was more difficult having to be performed under x ray control and requiring 2 incisions The first incision exposed the distal extremity and the second incision exposed the proximal extremity of the radius The nail was driven from the ankle joint upward to cross both the distal and proximal epiphyseal lines but not to penetrate the knee joint Closure of the 2 incisions was performed as described previously In this group the distal epiphyseal line of the radius not operated upon closed between the ages of 38 and 41 weeks, (an average of 39 5 weeks) while the proximal epiphyseal line closed sooner between the ages of 34 and 40 weeks (or an average of 37 weeks) In all of the radii operated upon the proximal epiphyseal line escaped early from the nail and remained open to maturity without deformity while the distal epiphyseal line retained the nail and closed at the average age of 27 weeks In addition the distal metaphysis and epiphysis showed marked deformity and angulation and the distal epiphyseal line closed about 14 weeks earlier than on the control side

In 3 animals in which the nail remained across the distal epiphyseal line to full bone maturity aged 41 weeks the total shortening of the radius in each was 25 per cent and in 2 animals (the third died) in which the distal epiphyseal lines escaped from the nail 3 and 8 weeks after insertion the total shortening at the completion of bone growth was 7 per cent and 12 per cent respectively

The fifth group of experiments was performed on a litter of 3 puppies at the age of 5 weeks as an operative control on the introduction of nails into the shaft of the radius without crossing the epiphyseal lines The technique of incision and closure was identical to that used in the first 2 series and the procedure was different only in that the Rush nail was withdrawn after it had been placed in the shaft of the right radius between the epiphyseal lines with care being taken not to injure the epiphyses The purpose of this control was to determine whether or not the operative procedure *per se* affected the growth of the bone At 10 weeks after the operation when 2 of the animals died and 5 weeks after the operation when the other animal died there was no difference in the length or size of the radius operated upon compared with the opposite radius not operated upon

The sixth group of experiments was performed on another litter of 3 pups also as an operative control but this time the nail was passed through the distal epiphyseal line into the shaft of the right radius and then withdrawn Otherwise the technique of the incision and the closure was exactly the same as that used in the fourth series The purpose of this control was to determine whether or not the operative procedure *per se* affected the growth of the epiphysis These operations were performed only recently and a final report cannot be given at this time but the x rays at 8 weeks showed no change between the surgical and non surgical radii

CONCLUSIONS

1 Intramedullary nails placed in the shaft of the radius of growing dogs do not affect the final longitudinal or transverse growth of the bone

or the time of closure of the epiphyseal lines, in relation to the opposite control radius

2 Intramedullary nails placed in the shaft of the radius of growing dogs and across the proximal and distal epiphyseal lines produce progressive deformity premature closure of the epiphyseal lines and shortening in proportion to the length of time the nail remains across the epiphyseal line

3 There is no difference between the intramedullary round Rush nail and the U shaped Kuentscher nail as pertains to the growth of the radius in growing dogs

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UTILIZATION OF RADIOACTIVE COBALT IN THE STERILIZATION OF HOMOGENEOUS BONE TRANSPLANTS*

PAUL H DEVRIES WADE O BRINKER AND LLOYD KEMPE

In the past year and a half there have been reported 4 cases of tuberculous wound infections and 1 case of homogulous serum hepatitis following the use of refrigerated bone bank bone[1 2] These 5 cases undoubtedly represent only *a small proportion of the postoperative wound infections* following bone grafting procedures but serve to emphasize the absolute necessity of transplanting sterile bone

Obtaining sterile bone at autopsy is a laborious time consuming chore that requires extra facilities and personnel and interdepartmental cooperation at all hours of the day and night Even under ideal conditions one can expect that approximately 20 per cent of the bone obtained at autopsy will be contaminated[3] and therefore unusable unless it is sterilized prior to transplantation

Although bacterial contamination of bone is rather easily detected by the usual bacteriological methods viral contamination of bone is almost impossible to detect and we rely solely on a negative history of jaundice when choosing bone donors in spite of the fact that donors of the hepatitis virus do not always have a history of jaundice or liver disease

Boiling and autoclaving bone prior to its use in all probability destroys the bacterial and viral contaminants but has the disadvantage of denaturing the protein constituents and inactivating the tissue enzymes Freeze drying bone although an excellent means of preservation does not ensure the surgeon of a sterile product for it is recognized that many bacteria and viruses including the common pathogens are resistant to freezing and freeze drying

In the past few years high speed electrons and radioactive isotopes have been utilized to improve diagnosis and treatment as well as to prepare tissues for transplantation Meeker and Gross[4] have reported the transplantation of irradiated aortas and Kreuz and Hyatt[3] have stated that the feasibility of cathode ray sterilization of bone bank bone was being investigated by their group More recently MaCris[5] reported the experimental

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transplantation of freeze dried aortas which had been sterilized by the gamma radiations from radioactive cobalt^{60}

It is known that high intensity gamma radiation from 10 000 curie radioactive cobalt source will inactivate certain bacteria and viruses and that it is useful in the preservation of meat and other food products It has been found that certain enzymes are only slightly altered by relatively large doses of gamma radiation[6] and parenteral fluids have been sterilized by gamma radiation and administered to animals without harmful effects[7] It therefore seemed worthwhile to conduct some experimental procedures to determine if bone could be sterilized by exposure to radioactive cobalt without destroying its ability to stimulate new bone formation

METHOD

Segments of dog humerus obtained without sterile precautions were purposely contaminated with a 1 day old culture of Clostridium tetani and 2 of the Clostridium botulinum organisms The contaminated bone was then sealed in glass or plastic tubes and subjected to different levels of high intensity gamma radiation from a 10 000 curie radioactive cobalt source The anerobic spore forming organisms were chosen as contaminants since they have been shown to be much more resistant to radiation sterilization than the vegetative nonspore forming organisms After irradiation the contaminated bone was subcultured in Brewer s thioglycolate media to determine which dosage level consistently produced sterile cultures

To determine if radiation alone would serve as a means of bone preservation segments of irradiated dog rib were stored at room temperature at 4°C and at −15°C for a period of 6 months Other segments of dog rib were subjected to a sterilizing dose of gamma radiation and used for the transplantation experiments These segments of dog rib were obtained without sterile precautions and were stored at room temperature from 1 to 25 days following irradiation before they were transplanted The control bone in the transplantation experiments consisted of dog rib obtained in the same fashion as the test rib but was not irradiated prior to transplantation The control segments were transplanted within 24 hours from the time they were obtained

Transplantation of test and control bone was accomplished by excising approximately ½ to 1 inch of the mid ulnar shaft and filling the defect with split pieces of rib No attempt was made to reapproximate the periosteum and no external splints were used to support the limb Aseptic operative techniques were used in all test and control procedures and none of the animals received antibiotics before or after the operation Twenty ulnas in 18 dogs received irradiated rib and 5 ulnas in 5 dogs received control rib

RESULTS

It was found that all bone receiving 2 000 000 rep (roentgen equivalent physical) and over was bacteriologically sterile Grossly there was no apparent change in the character of the bone immediately following irradiation and microscopic examination of the irradiated rib revealed only minimal postmortem changes in the marrow Irradiated bone stored at room temperature for 6 months however showed complete postmortem loss of the bone marrow and focal areas of calcium crystal deposition in the marrow



Fig 1 Irradiated rib transplant 10 days and 4 months postoperative

Fig 2 Irradiated rib transplant 2 weeks and 9 weeks postoperative

Fig 3 Irradiated rib transplant 1 week and 10 weeks postoperative

spaces No change was observed in the bony trabeculae other than the gradual disappearance of the cells from their lacunae and an increasing uptake of the eosin stain Refrigeration of the irradiated bone at 4 C and –15°C only retarded and did not prevent the gradual loss of the marrow Similar changes were also noted in nonirradiated rib stored at 4°C and –15°C and served to demonstrate that refrigeration alone does not prevent the gradual deterioration of bone tissue

In the 18 test animals all 20 operative wounds healed primarily No unusual systemic reactions were observed immediately following the transplantation procedures and all animals used the operated limb 2 to 3 days following the procedure X ray examination of the transplant sites at periodic intervals revealed the gradual disappearance of the transplants and the formation of new bone within the gap in all but 3 In 2 of these 3 not demonstrating new bone formation there was observed gradual disappearance of the transplant and atrophy of the distal ulnar fragment In one transplant there was no evidence of bony union at either end but the transplant had not disappeared and had the appearance of viable bone by x ray

Of the 5 control animals 2 developed a severe wound infection and a generalized toxic reaction necessitating sacrifice on the 10th postoperative day Two developed a mild wound infection with abscess formation but after incision and drainage of the abscess healing progressed to a point of union at the proximal end within 5 months In the remaining control animal there were no signs or symptoms of wound infection and satisfactory union occurred at the proximal end in 4 months

DISCUSSION

Although the level of gamma radiation which was found to be effective in destroying bacterial contaminants of bone was 2 000 000 rep this level will probably not prove to be the minimum safe level for sterilization of all bone deposited in a bone bank Similar experiments utilizing viral contaminants of bone in rats have demonstrated that between 3 000 000 and 4 000 000 rep are necessary to inactivate viral contaminants of bone[7]

The examples of nonunion and delayed union in the test animals were not interpreted as a fault of the radiation sterilization but were attributed to inadequate immobilization of the ulnar fragments The ulna in the dog is a nonweight bearing bone and it is quite likely that the lack of functional stress and strain played a part in the atrophy of the distal ulnar fragments in 2 animals

CONCLUSIONS

Experimental procedures demonstrate that relatively high levels of gamma radiation from a 10 000 curie radioactive cobalt source will render bone bacteriologically sterile Radiation alone, however will not serve as a means of bone preservation and other methods of preservation such as freezing or freeze drying must be used to prevent deterioration Irradiated bone can be transplanted into dogs without producing any immediate harmful effects and our results indicate that radiation sterilization of homogenous bone does not destroy its ability to stimulate new bone formation

REFERENCES

1 James J I P Tuberculosis transmitted by bank bone J Bone Surg Brit Vol *35 B* 678 1953
2 Shutkin N M Homologous serum hepatitis following the use of refrigerated bone bank bone J Bone Surg Brit Vol *36 A* 160 1954
3 Kreuz F P Hyatt G W Turner T G and Bassett C A L Use of preserved tissues in orthopedic surgery A M A Arch Surg *64* 148 1952
4 Meeker I A Jr and Gross R E Low temperature sterilization of organic tissue by high voltage cathode ray irradiation Science *114* 283 1951
5 MaCris J A The use of cobalt as a sterilizing agent for aortic homografts I Effect of gamma ray irradiation upon the structural integrity of the graft in Surgical Forum 1954 Philadelphia W B Saunders Co 1955
6 Michigan Memorial Phoenix Project Progress Report No I (Summary) October 1952
7 R T Jordan Personal communication

THE CLINICAL USE OF CATHODE RAY STERILIZED GRAFTS OF CADAVER BONE*

A Preliminary Report

C Andrew L Bassett Thomas F Hudgins Jr John G Trump and Kenneth A Wright

At the New York Orthopaedic Hospital the demand for preserved bone has often outstripped the supply available from the operating rooms It therefore became desirable to seek an additional source of bone Procurement of tissues from cadavers has been utilized with success by Hyatt and his associates at the Navy s tissue bank[1] However even with the advantages of special operating room facilities the Navy group has reported graft rejection rates as high as 16 per cent due to bacterial contamination[2] Our contam

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ination rate has approached 100 per cent for bone grafts procured with aseptic precautions from cases in the autopsy room The control of sterility of bone obtained at autopsy posed such a problem that this potential source could not be utilized at the onset of the project

In 1951 Meeker and Gross[3] reported the successful sterilization of arterial grafts by cathode rays Concomitant to this arterial work Hyatt s group began an investigation of the feasibility of cathode sterilization of bone[4] Turner in the Navy group[5] and Cohen,[6] working independently demonstrated the effectiveness of cathode rays in sterilizing experimental bone grafts Radiated bone was found to be incorporated by the host in a manner and rate comparable to nonradiated bone Recently DeVries[7] has reported the successful sterilization and host incorporation of experimental bone grafts subjected to Cobalt 60 radiation These encouraging results stimulated us to test cathode ray sterilization of bone in a clinical experiment

METHODS

Cadavers below the physiologic age of 60 years were deemed satisfactory if they had no evidence of malignant or viral diseases In addition bone had to show no signs of chronic metabolic changes or high bacterial concentrations Permission was obtained from the next of kin Autopsy permits specifically stated that removed tissues could be used for therapeutic purposes Bone was collected within 21 hours of death from bodies refrigerated to 3°C within the first hour following death

In the autopsy room, chest iliac crest and antero lateral tibial regions were washed and shaved Towels were used to demarcate the operative fields Clean cap mask gown gloves and instruments were used for tissue removal and processing Contamination with foreign materials such as hair was avoided Ribs iliac crests tibial strips and fibular shafts were cut into appropriate sizes measuring no thicker than 1 5 cm Sharp bone edges were removed Individual grafts were heat sealed* within 3 layers of 003 inch Lay Flat polyethylene tubing † Pooled bone samples from each graft were separated into 2 portions One portion was cultured immediately and the other sealed in polyethylene These sealed bacterial control packages containing about 15 samples of bone apiece were frozen and radiated with each group of grafts Initial freezing and storage was effected in a unit operating at –35 to –40°C A crated thermos filled with dry ice was used to transport the grafts by Railway Express Special Handling to and from the irradiation source

The source of high energy electrons used in this study was a pressure insulated electrostatic generator of the Van de Graaff type at the Massachusetts Institute of Technology Irradiation was carried on with the bone close to the temperature of dry ice The grafts were placed on a conveyor belt and passed through the *3 000 000 volt electron treatment* beam the current being adjusted to deliver *2 000 000 rep* (roentgen equivalent physical) in an irradiation time of 10 seconds

Radiated grafts packed in dry ice were returned within 36 hours Once grafts were frozen they were not thawed until used at the operating table Bacterial control samples were cultured and found to be sterile before bone

*Hand Heat Sealer — Dobeckmun Co Cleveland Ohio

†Lay flat polyethylene tubing Chippewa Plastics Co Chippewa Falls Wisconsin

was released At time of use the graft deposit unit was removed from the deep freeze and immersed in 1 1000 zephiran until sterile The end of the outer envelope was removed with sterile scissors and the packaged graft handled aseptically

RESULTS

This preliminary report is based upon tissue removed from 16 autopsies An average of 11 5 hours elapsed between death and bone procurement Approximately 1 hour per case was required to collect 7 to 10 bone deposits Grafts were stored an average of 22 days prior to use after sterilization

Radiated grafts showed few grossly evident alterations However, cortical bone was yellower and cancellous bone was browner than could be expected from conversion of oxyhemoglobin to hemaglobin Grafts were more brittle than comparable nonradiated bone Alterations in elasticity were not evident in the operative use of radiated cortical grafts However cancellous H grafts used in lumbosacral fusions were occasionally fractured during shaping When necessary this partial loss of elasticity was reduced by soaking the radiated bone in warm sterile normal saline for 15 minutes

Hemolytic and nonhemolytic streptococci staphylococci Escherchia coli Clostridium welchii Bacillus subtilis Proteus vulgaris Micrococci and Aerobacter aerogenes have been cultured singly or in combination from preradiation bone samples Staphylococci identical with those culturable from the surfaces of the autopsy room were the most commonly isolated bacteria No organisms have been cultured from postradiation bone control samples In addition bone removed from each graft in the first autopsy series proved to be sterile at the time of graft use Random samples of bone removed from grafts at the operating table in a continuing sterility double check have been 100 per cent sterile

In our wound healing study 100 patients received 189 radiated grafts The follow up on this group is from 3 to 9 months with the average 6 8 months Two wound infections have occurred A series of 31 patients within the group of 100 has been followed from 6 to 9 months and has shown no untoward clinical or radiographic responses to radiated bone Consolidation of ground cancellous bone masses with the establishment of a trabecular pattern has been radiographically evident in 4 to 5 months Exploration of 2 patients has shown grafts to be united at host contact sites and to be well revascularized as judged by bleeding in 4 to 5 months after grafting Biopsies of 3 to 5 week old fusion masses were obtained at the second or third stages of spine fusions for scoliosis There has been no gross or microscopic evidence of graft rejection inflammatory response or foreign body reaction in these biopsies of 3 to 5 week old fusion masses

DISCUSSION

We prefer fresh autogenous bone as a graft material However a bone bank is maintained to provide homogenous grafts for cases where autogenous bone procurement is hazardous or impossible Freezing and freeze drying have proved the most satisfactory methods for storing bone Unfortunately for our purposes most bacteria are well preserved by both freezing and freeze-drying Recently James[8] has reported the transmission of tuberculosis by frozen bone

This report is preliminary because a complete clinical picture of graft

acceptance and incorporation cannot be given. It is therefore not known whether irradiated bone will have a higher grafting success rate than the relatively low rates of bone sterilized by boiling, autoclaving or merthiolate storage.[6] Certainly, alterations in a graft's chemical and physical composition may be responsible for failure to stimulate and support host bone formation. Fortunately, irradiation at dry ice temperatures dramatically reduces adverse tissue effects but does not materially increase the sterilization dose. Experimentally, the sterilizing dose has been below the level where graft physical-chemical changes alter host acceptance.[5, 6, 7]

A dose of 2,000,000 rep has been effective in uniformly sterilizing bone grafts contaminated with a wide bacterial spectrum. The destruction of organisms depends upon the type and concentration of bacteria as well as the electron dosage. The level of irradiation used provided a margin of safety considering the types and low concentrations of bacteria usually occurring in cadaver bone. Since 1,000,000 to 1,000,000 rep are required to inactivate most virus particles, precautions were taken to exclude cadavers harboring known pathogenic viri. Penetration of electrons in a material varies directly with the electron voltage and inversely with the density. With 3,000,000 volt electrons applied from opposing sides, bone samples up to 1.5 cm. in actual thickness or up to 2.5 cm. in water equivalent thickness can be sterilized throughout their volume.

The 2 wound infections occurring in this series were probably not attributable to graft contamination. In one case, infection was subcutaneous; cultures and biopsy of the graft area showed no evidence of infection. The organism cultured from the second case was not isolated in any of the donor's preirradiation specimens. This 2 per cent infection rate compares most favorably with the rates reported by others using comparable quantities of bank bone. In 1919 Weaver[10] reported an infection rate of 8.1 per cent, in 1951 Wilson[11] reported 1.5 per cent, and Bray[12] in 1953 reported a rate of 9.8 per cent.

Clinical evaluation of patients will continue until a definitive answer can be given on the effectiveness of cathode ray sterilized bone as a graft material. At the present, the method can be recommended only as a means for sterilizing bone.

CONCLUSIONS

(1) Contaminated cadaver bone can be effectively sterilized by cathode rays.

(2) The wound infection rate in a series of 100 cases was 2 per cent. Both cases responsible for this rate had infections probably not attributable to cathode ray sterilized grafts.

(3) Preliminary clinical and radiographic results tend to substantiate the experimental effectiveness of irradiated bone grafts demonstrated by other investigators.

REFERENCES

1. Hyatt, G. W., Turner, T. C., Bassett, C. A. L., Pate, J. W., and Sawyer, P. N.: New methods for preserving bone, skin and blood vessels. Postgrad. M. *12*: 239, 1952.
2. Turner, T. C., Bassett, C. A. L., Pate, J. W., Sawyer, P. N., and Kellum, W. F.: The use of preserved tissues in surgery. Naval Medical Research Institute Lecture and Review Series No. 52-2. Bethesda, Md., 1952.
3. Meeker, I. A., Jr., and Gross, R. E.: Sterilization of frozen arterial grafts by cathode ray irradiation. Surgery *30*: 19, 1951.

4 Kreuz F P Hyatt G W Turner T C and Bassett C A L Use of preserved tissues in orthopedic surgery A M A Arch Surg *64* 149 1952
Turner T C Bassett C A L Pate J W, Sawyer P N Trump J G and Wright K A Sterilization of preserved bone grafts by high voltage cathode irradiation J Bone Surg Am Vol (In press)
6 Cohen J Cathode sterilization of bone grafts A M A Arch Surg (In press)
7 DeVries P H Kempe L L and Brinker W O Sterilization of bone transplants by Cobalt-60 radiation Univ Michigan M Bull *21* 29 1955
8 James J I Tuberculosis transmitted by banked bone J Bone Surg Brit Vol *35* 578 1953
9 Reynolds F C Oliver D R and Ramsay R Clinical evaluation of merthiolate bone bank and homogenous bone grafts J Bone Surg Am Vol *33* 873 1951
10 Weaver J B Experiences in the use of homogenous (bone bank) bone J Bone Surg Am Vol *31* 778 1949
11 Wilson P D Follow up study of the use of refrigerated homogenous bone transplants in orthopaedic conditions J Bone Surg Am Vol *33* 307 1951
12 Bray E A A comparative clinical study of autogenous and frozen homogenous bone in grafting procedures Clin Orthop *3* 165 1954

OBSERVATIONS ON EPIPHYSEAL GROWTH POTENTIAL FOLLOWING TEMPORARY HYPERVITAMINOSIS A*

EDWIN G BOVILL JR

Bone is a favorable tissue for the study of growth as it involves alteration of form as well as increase in size Bone is also unique as a growing tissue in its separation of the cellular function of reproduction for replacement from reproduction of growth in size the latter function being delegated to the epiphyseal cartilage only Even circumferential growth of the shaft appears to be under the organizing influence of epiphyseal cartilage as seems implied by the work of Wolbach[1]

Wolbach[1] has demonstrated that excessive doses of vitamin A in the experimental young animal (guinea pigs rats puppies chicks) will produce rapid consumption of the epiphyseal cartilage This rapid consumption can be carried to completion and ossification of the epiphyseal plate in a period of time as short as 10 to 15 days The rate of change varies with the dosage of vitamin A and partial consumption can also be obtained I have found no experimental work to demonstrate the subsequent growth potential of such partially consumed epiphyseal plates This paper is the report of such an investigation as the first step in the further evaluation of the organizer effect of epiphyseal cartilage on various diseased states in bone

METHOD

Guinea pigs were the animals used The availability of the age order of union in epiphyseal plate of this animal as reported by Zuck made the guinea pig the animal of choice Two epiphyseal plates were chosen for observation The distal humeral epiphysis as an example of an early closing plate and the distal femoral epiphysis as an example of a late closing epiphysis

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This work was carried out under a grant from the American Cancer Society



Fig 1



Fig 2



Fig 3



Fig 4

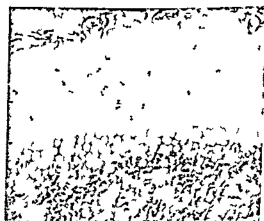


Fig 5

All animals were begun at close to 3 weeks of age and except for controls received from 5 to 6 days of daily consecutive intramuscular injections of sterile vitamin A in oil The preparation was concentrated to 250 000

international units/cc and the animals received from 500 to 1000 units per gm of body weight No detectable histologic differences were noted between those receiving 500 units and those receiving 1000 units so long as the duration was 5 to 6 days only Wolbach has already demonstrated that 1250 units per gm for 10 to 15 days would obliterate the epiphyseal plate and usually be followed shortly by death from inanition and secondary infection A total of 16 animals were used equally divided as to sex Six were utilized for humeral observation 10 for femoral observation The animals were sacrificed at staggered intervals from 4 weeks to 18 weeks of age Sagittal sections were made through the epiphyses and stained with hematoxylin and eosin stain The histologic features of reaction to vitamin A in excess were identical with those previously described by Wolbach[1]

Figure 1 is the distal humeral epiphysis of a 4 weeks old guinea pig that had received no medication Figure 2 is the same epiphysis of a litter mate that had received 1000 units per gm of vitamin A by injection daily during its 4th week of life and was then sacrificed The difference in thickness is apparent Distal humeral epiphyseal plates of all treated guinea pigs closed in 5 to 7 weeks as compared to the normally anticipated 9 to 10 weeks

Figure 3 is the distal femoral epiphysis of a 4 weeks old control animal Figure 4 is the same epiphysis of a 4 weeks old litter mate treated as outlined with 1000 units per gm of vitamin A during its 4th week of life The difference in thickness is again apparent Figure 5 is the distal femoral epiphyseal plate of a guinea pig 11 weeks of age that had received the prescribed dosage of vitamin A for 6 consecutive days during its 4th week of life Note that now the epiphysis has regained its normal architecture and that the zone of degenerated cartilage which was absent in Figure 4 has been reformed This is indistinguishable from the untreated epiphyseal plate at a comparable age The 2 distal femoral epiphyses followed to 18 weeks of age the anticipated date of beginning closure were normal to histologic examination None of the animals died or suffered any ill effects from the dosages used

No discrepancy in femoral length was observed

This evidence makes it reasonable to assume that partial consumption of epiphyseal cartilage in the guinea pig as a result of hypervitaminosis A can be followed by resumption of normal growth in both space and time However if the epiphyseal plate is approaching maturity and closure when exposed to the vitamin as the distal humeral examples here resumption of normal growth may not ensue

REFERENCES

1 Wolbach S B Vitamin A deficiency and excess in relation to skeletal growth J Bone Surg Am Vol 29 171 192 1947

2 Zuck T T Age order of epiphyseal union in guinea pig Anat Rec 70 389 399 1938



Fig 1



Fig 2



Fig 3



Fig 4

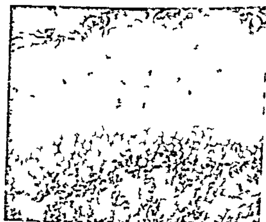


Fig 5

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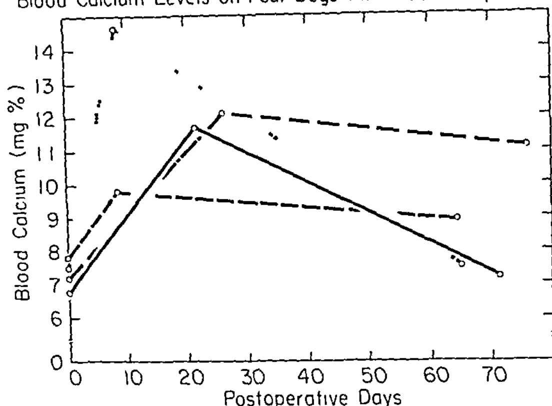


Fig 1

Table 1 The Fate of Plaster of Paris Columns Inserted Into the Dog's Radius

NO	SIZE OF DEFECT	DAYS PRIOR TO SACRIFICE	ABSORPTION OF PLASTER	BONE FORMATION	REMARKS
			COMMERCIAL PLASTER		
1	4 0 cm	117	complete	absent	
2	1 5 cm	82	complete	absent	
3	4.5 cm	96	complete	present	complete regeneration
4	3 7 cm	98	complete	present	complete regeneration
5	3 7 cm	117	complete	absent	
6	4.3 cm	59	complete	present	partial regeneration
7	3 0 cm	65	complete	present	partial regeneration
8	3.5 cm	28	partial	absent	
9	4 2 cm	28	partial	present	beginning regeneration
10	4 2 cm	30	partial	present	excessive callus
			DENTAL PLASTER		
11	2 5 cm	92	complete	present	partial regeneration
12	2 8 cm	108	complete	present	complete regeneration
13	2.5 cm	45	complete	absent	sepsis no regeneration
14	4 0 cm	66	complete	absent	

THE SUBSTITUTION OF PLASTER OF PARIS RODS FOR PORTIONS OF THE DIAPHYSIS OF THE RADIUS IN DOGS*

LEONARD F. PELTIER AND ROBERT LILLO

In April 1953 Kovacevic a surgeon in Sarajevo described the use of plaster of Paris to replace very large defects in the tibia of patients suffering from acute hematogenous osteomyelitis The defects were produced by diaphysectomies carried out in the treatment of these infections Successful control of infection and regeneration of bone resulted in the 3 cases which he reported Intrigued by his paper, we decided to carry out experiments in the dog to determine whether or not similar results could be obtained in the absence of infection

METHOD

Small mongrel dogs of approximately 1 year of age were anesthetized with sodium pentobarbital and a tourniquet was applied to one of the fore limbs After preparing the skin an incision was made over the radius the periosteum was incised and stripped from a portion of the bone and a segment of the diaphysis completely excised The periosteum was very thin and was usually badly shredded during the procedure Into the defect was placed a column of plaster The wound was closed with several fine catgut sutures in the muscle and with silk sutures in the skin A compression dressing was applied before removing the tourniquet A tongue blade was incorporated into the dressing for support At no time were antibiotic drugs given to these animals The dressings were removed at the end of 3 weeks

The plaster columns were made by pouring plaster of Paris ($CaSO_4$) into paper tubes (2 cm x 10 cm) and allowing time for hardening The paper was then removed and the columns sterilized by dry heat in an oven at 200°C for 48 hours At operation the columns were shaved to the dimensions of the resected bone Two types of plaster of Paris were used (1) the commercial grade and (2) the finer dental grade used for making dental moldings

Roentgenograms were made at the end of the operation and at intervals until the completion of the experiments

On some of the animals blood for calcium determinations was drawn prior to operation and at intervals during the postoperative period The calcium level in the blood was determined by Clark and Collip s modification of Tisdall s method [1]

Columns of commercial plaster of Paris were placed in 10 dogs of dental plaster of Paris, in 4 dogs

After sacrificing the animals the final roentgenograms were made The radii were fixed in formalin Sections through the area of the transplant were stained with hematoxylin and eosin

DISCUSSION

The size of the diaphyseal portion resected in these animals was sufficient to preclude spontaneous regeneration [2,4,5] The size of the defect itself

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HEALING OF FRACTURES IN DENERVATED LIMBS IN RATS*

William S. Smith and Ensor R. Dunsford, Jr

The purpose of this study is to determine the effect of denervation of limbs on the healing of fractures, with particular reference to the associated osteoporosis attendant with motor denervation. Since any condition that will prevent use of an extremity will eventuate in atrophy of both soft tissue and bone, motor denervation has been selected as the first of such conditions to be subjected to critical investigation. The merits of being able to study the healing of fractures in animals of uniform age, diet and with selective osteoporosis hardly requires elaboration. And by the same token a study of the adequacy and rapidity of the reparative process in bone in clinical practice is practically impossible because of the marked variation of circumstances accompanying fractures in humans.

Atrophy of bone in dogs following femoral and sciatic section was noted as early as 1851 by Schiff. Since then numerous investigations of the changes in bone following motor and sensory denervation have been carried out.[1,2,4,5,6] In one of the most significant studies Allison and Brooks[1] showed in dogs that the changes in bone were the same after section of the brachial plexus, excision of the proximal end of the humerus, or plaster fixation. Such changes consisted of a decrease in the diameter of the shaft, an increase in the diameter of the medullary canal, diminished cortical thickness and smaller and fewer trabeculae. In the past 3 decades investigators agree that the changes in the bone following disuse from any cause are primarily quantitative with no changes in the character of the matrix but rather that there was simply less matrix.

Historically, one more aspect of limb denervation requires further elaboration, that is, the concept that bone atrophy is regulated by nervous influences. Sensory nerves have been sectioned by many workers[3,4,7] without producing bone atrophy and no sensory nerves have been demonstrated entering bone itself. Gillespie[5] found a direct proportion between the atrophy of the muscle and the atrophy of the bone. Numerous references can be found to the bone ash content following motor denervation, the exact percentage decrease varying from 10 to 20 per cent.[4,5]

METHOD

In the present experiment 46 white rats of the Sprague Dawley strain were used. The diet was the same in all cases. A section of the sciatic nerve 6 to 10 mm. was removed in 18 of the animals within a 2 weeks period. The right tibia and fibula of each animal were fractured under general anesthesia 35 days after partial sciatic resection. On the same date the tibiae and fibulae of 15 normal control rats were fractured manually.

Sciatic avulsion was performed on 4 additional rats which were sacrificed with 4 control animals 35 days later. The tibiae and fibulae were dissected, cleaned and subjected to roentgen ray examination. Although changes in density of the bone have been noted roentgenologically following denervation in rats after this period (Gillespie), we were unable to confirm his

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appeared to have no relationship to the occurrence of bony regeneration associated with the plaster insert.

The time required for completion of the regenerative process in the radius of the dog was about 3 months. There was complete disappearance of the plaster column as determined by roentgen examination, in all animals followed 15 days or more. The disappearance of the plaster was accompanied by a rise in the blood calcium level. This level returned to normal as the plaster became completely absorbed. There appeared to be no difference in the behavior of plaster of Paris of commercial or dental grade.

Evidence of new bone formation was apparent on roentgenograms and in histological sections in 8 of the 11 animals. Initially there appeared to be a large number of giant cells about the plaster particles. Later on, the cells characteristics of actively healing bony callus appeared, i.e., cartilage cells, fibroblasts and osteoblasts. *Three of 7 dogs followed for 90 days or more* showed complete bony restitution, one partial regeneration. Of the remaining 7 animals followed less than 90 days, actively progressing regeneration was present in 4.

Infection was a complication in only 1 of the dogs. In this instance there was complete disappearance of the plaster insert.

CONCLUSIONS

The insertion of plaster of Paris ($CaSO_4$) columns into diaphyseal defects in the radii of dogs is followed by an absorption of the plaster, accompanied by an elevation of the blood calcium level. In 8 of 11 animals there was good evidence of bony regeneration with complete restitution of the diaphyseal defect in 3.

REFERENCES

1. Clark, E. P., and Collip, J. B.: A study of the Tisdall method for the determination of blood serum calcium with a suggested modification. J. Biol. Chem. *63*: 461-464, 1925.
2. Key, J. A.: The effect of a local calcium depot on osteogenesis and healing of fractures. J. Bone Surg. Am. Vol. *16*: 176-184, 1934.
3. Kovacevic, B. I.: Ein Beitrag zum Problem der hämatogenen Osteomyelitis. Deut. Ztschr. Chir. *276*: 432-443, 1953.
4. Murray, C. R.: The repair of fractures. Minnesota M. *13*: 137-153, 1930.
5. Schram, W. R., and Fosdick, L. S.: Studies in bone healing. J. Oral Surg. *1*: 191-196, 1943.

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William S. Smith and Ensor R. Dunsford Jr.

The purpose of this study is to determine the effect of denervation of limbs on the healing of fractures with particular reference to the associated osteoporosis attendant with motor denervation. Since any condition that will prevent use of an extremity will eventuate in atrophy of both soft tissue and bone, motor denervation has been selected as the first of such conditions to be subjected to critical investigation. The merits of being able to study the healing of fractures in animals of uniform age, diet, and with selective osteoporosis hardly requires elaboration. And by the same token, a study of the adequacy and rapidity of the reparative process in bone in clinical practice is practically impossible because of the marked variation of circumstances accompanying fractures in humans.

Atrophy of bone in dogs following femoral and sciatic section was noted as early as 1851 by Schiff. Since then, numerous investigations of the changes in bone following motor and sensory denervation have been carried out.[1, 4, 5, 6] In one of the most significant studies, Allison and Brooks[1] showed in dogs that the changes in bone were the same after section of the brachial plexus, excision of the proximal end of the humerus, or plaster fixation. Such changes consisted of a decrease in the diameter of the shaft, an increase in the diameter of the medullary canal, diminished cortical thickness, and smaller and fewer trabeculae. In the past 3 decades investigators agree that the changes in the bone following disuse from any cause are primarily quantitative with no changes in the character of the matrix, but rather that there was simply less matrix.

Historically, one more aspect of limb denervation requires further elaboration, that is, the concept that bone atrophy is regulated by nervous influences. Sensory nerves have been sectioned by many workers[3, 4, 7] without producing bone atrophy, and no sensory nerves have been demonstrated entering bone itself. Gillespie[5] found a direct proportion between the atrophy of the muscle and the atrophy of the bone. Numerous references can be found to the bone ash content following motor denervation, the exact percentage decrease varying from 10 to 20 per cent.[4, 5]

METHOD

In the present experiment, 16 white rats of the Sprague Dawley strain were used. The diet was the same in all cases. A section of the sciatic nerve 6 to 10 mm. was removed in 18 of the animals within a 2 weeks period. The right tibia and fibula of each animal were fractured under general anesthesia 35 days after partial sciatic resection. On the same date, the tibiae and fibulae of 15 normal control rats were fractured manually.

Sciatic avulsion was performed on 4 additional rats which were sacrificed with 4 control animals 35 days later. The tibiae and fibulae were dissected, cleaned, and subjected to roentgen ray examination. Although changes in density of the bone have been noted roentgenologically following denervation in rats after this period (Gillespie), we were unable to confirm his

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appeared to have no relationship to the occurrence of bony regeneration associated with the plaster insert

The time required for completion of the regenerative process in the radius of the dog was about 3 months There was complete disappearance of the plaster column as determined by roentgen examination in all animals followed 45 days or more The disappearance of the plaster was accompanied by a rise in the blood calcium level This level returned to normal as the plaster became completely absorbed There appeared to be no difference in the behavior of plaster of Paris of commercial or dental grade

Evidence of new bone formation was apparent on roentgenograms and in histological sections in 8 of the 14 animals Initially there appeared to be a large number of giant cells about the plaster particles Later on the cells characteristics of actively healing bony callus appeared i e cartilage cells fibroblasts and osteoblasts Three of 7 dogs followed for 90 days or more showed complete bony restitution one partial regeneration Of the remaining 7 animals followed less than 90 days actively progressing regeneration was present in 4

Infection was a complication in only 1 of the dogs In this instance there was complete disappearance of the plaster insert

CONCLUSIONS

The insertion of plaster of Paris ($CaSO_4$) columns into diaphyseal defects in the radii of dogs is followed by an absorption of the plaster accompanied by an elevation of the blood calcium level In 8 of 14 animals there was good evidence of bony regeneration with complete restitution of the diaphyseal defect in 3

REFERENCES

1 Clark E P and Collip J B A study of the Tisdall method for the determination of blood serum calcium with a suggested modification J Biol Chem *63* 461-464 1925

2 Key J A The effect of a local calcium depot on osteogenesis and healing of fractures J Bone Surg Am Vol *16* 176 184 1934

3 Kovacevic B L Ein Beitrag zum Problem der hamatogenen Osteomyelitis Deut Zschr Chir *276* 432-443 1953

4 Murray C R The repair of fractures Minnesota M *13* 137 153 1930

5 Schram W R and Fosdick L S Studies in bone healing J Oral Surg *1* 191 196 1943



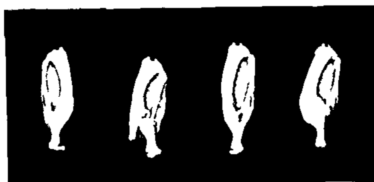


Fig 3 Forty three days after fracture (Controls in bottom row) Diminished density of the callus in the denervated group is again noted Osteoporosis of the entire bone following denervation is still more clearly evident The fracture line has not disappeared in the denervated group

35 days along with 5 controls of the same age One control fracture was compounded and the animal was discarded These animals were sacrificed after 43 days

RESULTS

Marked overriding of the fractures was observed in the control groups but to a much less degree in the denervated animals indicating the lack of muscle spasm as a factor in the denervated group In the 4 8 12 and 21 day postfracture groups no microscopic or roentgenographic changes could be ascertained in comparison to the denervated and control groups In the animals sacrificed 31 days after fracture x ray examination clearly showed the fractures to be healed with almost complete obliteration of the fracture line and the density of the callus simulating that of the parent bone However in the denervated group the fracture line was clearly visible and the density of the callus was clearly less than the parent bone In H and E stained sections of the fracture sites from both groups no qualitative differences were noted

In the animals sacrificed 13 days after fracture and 78 days after sciatic avulsion the changes were very similar to those noted in the 31 day group with the exception of further maturity of the healing process by x ray The differences in the density of the callus in the denervated group was again clearly less than the parent bone while the density of the callus in the control group was about the same as the parent bone The fracture line was clearly evident in the denervated group after 43 days while it had disappeared completely in the control group with the exception of 1 animal

finding after this period of time Densigraphic studies were not performed

The animals with fractured limbs were then sacrificed in 4 8 12 21 and 31 days These intervals were selected in order to determine any change in the initiation of the reparative process as well as in the completion of this process

Because of the roentgenographic appearance of delayed union after 31 days, 8 additional 8 weeks old rats of the same strain were subjected to denervation in the above manner The right tibia was again fractured after

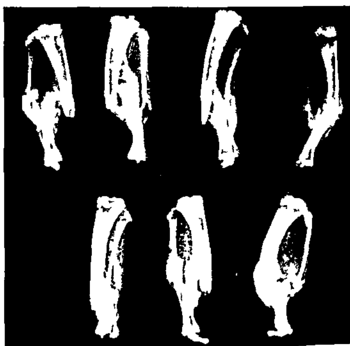


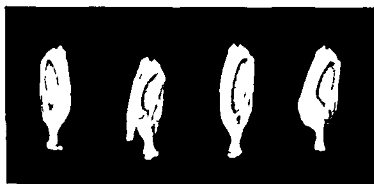
Fig 1 Twenty days after fracture Denervated specimens are in the top row while the controls are below There are no significant differences in this group with the exception of the atrophy noted in the denervated group



Fig 2 Thirty one days after fracture Note the decreased density of the callus in the denervated group (top row) and the persistent fracture line Osteoporosis of the tibia is now clearly evident



Fig 3 Forty-three days after fracture (Controls in bottom row) Diminished density of the callus in the denervated group is again noted Osteoporosis of the entire bone following denervation is still more clearly evident The fracture line has not disappeared in the denervated group



35 days along with 5 controls of the same age One control fracture was compounded and the animal was discarded These animals were sacrificed after 43 days

RESULTS

Marked overriding of the fractures was observed in the control groups but to a much less degree in the denervated animals indicating the lack of muscle spasm as a factor in the denervated group In the 4, 8, 12 and 21 day postfracture groups no microscopic or roentgenographic changes could be ascertained in comparison to the denervated and control groups In the animals sacrificed 31 days after fracture x-ray examination clearly showed the fractures to be healed with almost complete obliteration of the fracture line and the density of the callus simulating that of the parent bone However in the denervated group the fracture line was clearly visible and the density of the callus was clearly less than the parent bone In H and E stained sections of the fracture sites from both groups no qualitative differences were noted

In the animals sacrificed 43 days after fracture and 78 days after sciatic avulsion the changes were very similar to those noted in the 31 day group with the exception of further maturity of the healing process by x-ray The differences in the density of the callus in the denervated group was again clearly less than the parent bone while the density of the callus in the control group was about the same as the parent bone The fracture line was clearly evident in the denervated group after 43 days while it had disappeared completely in the control group with the exception of 1 animal

in which there was so much overriding that compounding had almost occurred. Again no qualitative changes were noted in the H and E stained sections of the latter groups. In both the 31 and 43 day postfracture denervated groups the osteoporosis was now clearly evident in the x-ray examinations compared to the control animals.

DISCUSSION

We cannot be oblivious to other factors involved in denervated limbs before concluding that delayed union of fractures in bone rendered atrophic by denervation results solely from the accompanying osteoporosis. Some of these factors are: (1) decrease in the calibre of vessels in the denervated parts, (2) decrease in the amount and character of the soft tissue, chiefly muscle, surrounding the fracture, (3) the marked displacement of the control fracture compared with the denervated limb, and (4) while the limbs of the entire group were continuously used once the fracture became firm, use of the fractured denervated limb was constantly curtailed by the effects of the denervation. To obviate these factors, it is planned to study the fractures in tenectomized limbs and arthrodesed or crushed joints.

As to the significance of the roentgen findings in the denervated group, we are of the opinion that the decreased density of the callus may well represent a quantitative reduction of the callus which is probably proportional to the reduction of the mass of bone in osteoporosis. There is little reason to expect a bone of diminished mass to elaborate a reparative process comparable to the same bone with a greater mass.

CONCLUSIONS

1. In rats, tibiae fractured 35 days after partial sciatic resection showed evidences of delayed union compared to normal controls.

2. Thirty-one days after fracture the main roentgenographic change, compared to the control, was persistent fracture line with decreased density of the calcified callus compared to the parent bone.

3. Forty-three days after fracture the delayed union was still evident.

4. Objections to the assignment of bones rendered atrophic by denervation as a means of studying the repair of fractures in the presence of osteoporosis have been outlined.

REFERENCES

1. Allison, N., and Brooks, B.: Bone atrophy. Surg. Gyn. Obst., *33*: 250-260, 1921.
2. Armstrong, W. D., Knowlton, M., and Couze, M.: Influence of estradiol and testosterone proprionate in skeletal atrophy from disuse and in normal bones of mature rats. J. Endocr. Lond., *36*: 313-322, 1945.
3. Corbin, K. B., and Hinsey, J. C.: Influence of the nervous system in bone and joints. Anat. Rec., *75*: 307-317, 1939.
4. Gillespie, J. A.: The nature of the bone changes associated with nerve injuries and disuse. J. Bone Surg., Surg. Brit. Vol., *36B*: 464-473, 1954.
5. Gillespie, J. A.: The influence of sex hormones in the boney changes occurring in paralyzed limbs. J. Endocr., *11*: 66-70, 1954.
6. Grey, E. G., and Carr, G. L.: An experimental study of the factors responsible for non-infectious bone atrophy. Bull. Johns Hopkins Hosp., *26*: 381-385, 1915.
7. Hurrell, Daniel J.: The nerve supply of bone. J. Anat. Lond., *72*: 54-61, 1937.

Plastic Surgery

INTRODUCTION

BRADFORD CANNON

Much of the experimental activity of the plastic surgeon in recent years has been directed toward a greater understanding of the nature of the problems of homotransplantation. The last few Forum sessions have reflected this interest with a number of papers each year on the subject. This year is no exception with 8 papers concerned directly or indirectly with the problem.

Parabiosis, transplantation of limb buds and primitive eyes in cold blooded animals, and the grafting of vessels and organs have been standard experimental techniques in lower animals for several decades. But permanent survival of homografts in adult animals and man has been possible only between identical twins. One well documented report of successful skin grafts between identical twins was published by Brown (1937). Corroborative reports by other authors have appeared in the literature since that time, including one in which successful exchange of skin grafts was accepted as a legal proof of identity.

Current studies on the nature of individual tissue specificity were stimulated by the important observation of Medawar (1943) that a second skin graft from the same donor to the same recipient was rejected more rapidly than the first. This evidence of a specific sensitization of the recipient by the donor tissue and its antigen antibody like reaction in the rejection of the transplant has led to a series of further investigative projects on both sides of the Atlantic. These have included the development of techniques for direct visualization and photographic recording of the microscopic changes in the graft and adjacent host tissues from the time of transplantation to the time of rejection, the establishment of objective methods for determining more accurately the end point of survival of a graft, studies on altering the host antibody response or the antigenicity of the transplant by cortisone or ACTH, antihistaminic drugs, radiation, splenectomy, or blocking the reticulo-endothelial system, and production of acquired tolerance by intrauterine injection of the fetus with homologous cells to establish a tissue tolerance similar to the natural phenomenon observed in dizygotic freemartin cattle between which both skin and kidney have been transplanted successfully.

The recent report of prolonged survival of human skin homografts to recipients who because of agammaglobulinemia are incapable of producing antibodies is further evidence that the intolerance of the normal recipient to foreign skin is related to an antigen antibody reaction. It also corroborates the clinical observation that in a seriously debilitated burned patient the duration of survival of the skin homograft is prolonged.

The value of the skin homograft as a temporary covering in the severely burned is well known In order to have skin available for the emergency situation the establishment of skin banks has been proposed A flawless method for prolonged preservation of skin and other homologous tissues has not been found but studies are being carried on and have been reported at Forum sessions in recent years concerning methods of preservation sterilization and storage of these tissues

COMMON ANTIGENICITY BETWEEN SKIN GRAFTS AND TOTAL LUNG TRANSPLANTS*

CREIGHTON A. HARDIN

The operative technique of transplanting one entire lung has been demonstrated to be feasible.[1,2,3] The limited survival of a transplanted lung, however, leaves unsolved the basic mechanisms involved in the rejection of a homograft.

Attempts to prolong the life of lung transplants by blocking the reticulo-endothelial system have proved unsuccessful. Benadryl, cortisone, total body irradiation[3] and splenectomy[4] all have failed to produce any appreciable difference in the survival of a homotransplanted lung as compared to the control group. In the group of animals in which the donor and recipient were inbred littermates the survival period of the transplanted lung was significantly longer.[3]

Dempster[5] has shown that a dog sensitized with a skin graft will react against a homotransplanted kidney. The same donor dog furnished the skin and kidney transplants. Conversely he showed that a skin graft on a dog which had been previously sensitized with a homotransplanted kidney will not survive as long as skin homografts do.

It became apparent that lung transplantation would lend itself nicely to the above experimental conditions in that a functional lung anastomosis could be done. Lung transplantation can be carried out with restoration of the pulmonary artery and vein and the bronchus. The only natural function not reconstituted would be the nerve supply.

METHOD

The surgical technique of lung transplantation used was the same as reported in 1952.[3] All operated dogs received 1 cc procaine penicillin daily during the period of observation.

A control series of 10 dogs were homografted with a skin dosage of 15 gm. The site of transplantation was the lateral thoracic wall. All skin grafts were sutured into a patterned surgical defect with interrupted 1-0 black silk stented sutures. After the first skin homografts sloughed a second skin homograft from the same donor was transplanted.

A one week time interval elapsed between the sloughing of the skin graft and the application of the second skin graft. Serial daily microscopic studies of the grafts were done in order to determine viability.

Another series of 7 dogs were homografted with a skin dosage of 20 gm. Again a one week period elapsed between the complete sloughing of the first skin graft and the transfer of the second skin homograft. Lung transplantation was then done 4 to 7 days after the sloughing of the second skin graft. The same donor animal provided the skin and lung homografts.

A third series of 8 dogs had lung transplants and the homografted lung was removed in from 2 to 7 days.

In 5 of the surviving animals a time interval of from 5 to 7 days elapsed before a homologous skin graft was applied. A group of skin grafts from another donor dog was used as a control.

*From the Department of Surgery, University of Kansas School of Medicine, Kansas City, Kans.

The value of the skin homograft as a temporary covering in the severely burned is well known. In order to have skin available for the emergency situation the establishment of "skin banks" has been proposed. A flawless method for prolonged preservation of skin and other homologous tissues has not been found, but studies are being carried on and have been reported at Forum sessions in recent years concerning methods of preservation, sterilization and storage of these tissues.

RESULTS

The survival of a first skin homograft (11 to 14 days) and a second skin homograft (1 to 5 days) closely parallels the observations of Dempster.[5]

The rapid destruction of a transplanted lung by previous sensitization with repeated skin homografts would seem to lend support to an antigen antibody reaction (Table 1). Conversely sensitization of a dog by a lung transplant and then its removal so sensitized the animal that the subsequent skin homograft was destroyed at a faster rate (Table 2).

Skin and lung as well as skin and kidney (Dempster) must therefore share important common antigens.

The use of skin and lung transplants does not lend itself to such a sharp end point as Dempster's[5] with skin and kidney grafts. His criteria of the length of urinary secretion of the homografted kidney was easy to determine. We used the length of survival of the lung transplanted dog previously sensitized with skin grafts. This group of animals showed the same pathological changes in the transplanted lung as compared to those of a control group.[4] The average length of survival of our control group of transplanted lungs was 5½ days.[4] Neptune and associates[6] reported an average survival of 9 days. The average length of survival of lung transplanted dogs previously sensitized with skin grafts was 2½ days (Table 2).

SUMMARY

1. A dog sensitized with a skin homograft will react against a lung transplanted from the same donor.

2. Skin homografts on a dog sensitized by a transplanted lung will not survive as long as skin homografts normally do.

3. It would then appear that lung and skin share a common individual immunity.

4. Support is thereby lent to the theory of an antigen antibody mechanism in the rejection of skin and lung homotransplants.

5. The basic problem of transplantation can perhaps be more easily and profitably studied in the behavior of factors governing skin homografts alone.

REFERENCES

1. Standacher, V. E., Belliannazzo, P., and Pullin, A.: Primary results in attempts at autoplastic reimplants and homoplastic transplants of pulmonary lobes. Chir. 5:233, 1950.
2. Davis, H. A., O'Connor, J. P., Colorviras, C. J., and Strawn, D. A.: Homotransplantation of lung. Arch. Surg. 61:745, 1952.
3. Hardin, C. A., Kittle, C. F., and Schafer, P. W.: Preliminary observations on homologous lung transplants in dogs. in Surgical Forum 1951. Philadelphia, W. B. Saunders Co., 1952, p. 374.
4. Hardin, C. A., and Kittle, C. F.: Experiences with transplantation of the lung. Science 119:97, 1954.
5. Dempster, W. J.: The relationship between the antigens of skin and kidney of the dog. Brit. J. Plastic Surg. 5:228, 1953.
6. Neptune, W. B., Redondo, H., and Bailey, C. P.: Experimental lung transplantation. in Surgical Forum 1951. Philadelphia, W. B. Saunders Co., 1952, p. 379.

Table 1 Skin and Lung Hemografts
Sensitization of the Dog by Means of Repeated Homografts

Survival in days of first skin homograft	Interval in days between sloughing & second skin homograft	Survival in days of second skin homograft**	Interval in days after sloughing before being transplanted	Days of survival of lung transplanted dogs
12	7	4	7	2
10	7	3	6	3
14	7	4	7	2
11	7	5	5	1
12	7	4	7	3
10	7	4	6	2
12	7	3	4	4

Dosage of skin 20gm Skin and lung transplants from same donor
* Surviving epithelium but none at 14 to 16 days
** 6th day

Table 2 Lung and Skin Transplants
Sensitization of the Dog by Means of a Lung Transplant

Days lung removed after transplant	Interval in days between removal of lung and skin homograft	Survival in days of skin homograft*	Control survival in days of skin grafted from another donor**
7	6	4	12
5	7	3	10
4	5	2	11
3	6	5	12
2	7	6	11
died 1st day p o	—	—	—
died 3rd day p o	—	—	—
died 4th day p o	—	—	—

Dosage of skin 20gm * Surviving epithelium but none beyond 7 days
** 14 16 days

Preservation and Transplantation Technique (Mouse) Grafts are excised from the depilated skin of the dorsum of the anesthetized adult mouse using aseptic technique Grafts varying from 1 to 8 mm are taken with the von Graefe knife and transferred to a sterile Petri dish containing balanced salt solution (Gey's solution or Hank's solution) Connective tissue and subcutaneous fat is removed from the grafts by dissection They are washed several times in balanced salt solution and transferred to a mica coverslip in a drop of homologous unheparinized plasma One part of homologous or heterologous (chick) embryo extract is added and clotting is allowed to proceed for 10 min at 37 C The mica coverslip is placed in a Pyrex test tube to which is added the maintenance media consisting of Hank's balanced salt solution 10 to 15 parts phenol red as indicator homologous serum 2 to 3 parts homologous placental extract 1 to 2 parts human cord serum 1 to 2 parts human carcinoma ascitic fluid 1 to 3 parts and dilute homologous embryo extract (1 100 or 1 500) 2 parts to make a composite of 20 parts The test tubes are placed in a roller drum and maintained at 10 revolutions per hour as designated by Gey and Gey[6] Occasionally Carrel D 3 5 mm flasks were employed instead of the Pyrex roller tubes Within 24 hrs the coagulum at the periphery of the explant is liquified and slow growth is noted Periodic explanation for 24 hrs to standard tissue culture media with high concentration of embryo extract provides a check of viability The maintenance media is completely replaced every 72 hrs and if the pH becomes too acid the specimen is transferred It is possible to maintain the grafts under these conditions for periods of 1 to 19 weeks prior to transplantation to a new host Seventy eight adult mice of both sexes different strains and different colors of coat (Swiss albino and C ,Br) have been used in the experiments reported herein This selection ensured maximum genetic diversity Preparation of the host and the site for the graft in the mouse was similar to that reported previously from this laboratory After the host was anesthetized the hair was removed by a depilatory cream and the dorsal fold of the skin was immobilized in a modified Joslin traction splint[7] Modifications included reduction of the arch of the splint to diminish the tension exerted on the soft tissues of the dorsum and the addition of 2 types of lateral outriggers to aid in maintaining the dorsal fold in an upright position so that angulation of the blood vessels did not occur In addition multiple grafts were applied to a single host This afforded a means of comparing microscopically the reaction of a single host to a fresh autograft to a homograft and to a heterograft each of which had been maintained *in vitro* for prolonged periods prior to transplantation This technique diminished the need for excessive numbers of animals since each animal served as its own control

Preservation and Transplantation Technique (Human) The technique of preservation of human skin was similar to that employed with experimental animals Specimens were excised and freed of subcutaneous tissue washed in balanced salt solution and mounted in plasma clots in Kohl flasks Maintenance media were the same as used with experimental animals except that human embryo extract was not available Specimens of skin for these experiments were obtained in the excision of decorative tattoos from the forearm or chest It was hoped that the tattooed specimens would offer

EFFECT OF MAINTENANCE *IN VITRO* ON THE SURVIVAL TIME OF HOMOLOGOUS SKIN GRAFTS*

Herbert Conway, Richard B Stark J D Sedar and A Alfred Lazzarini, Jr

The rejection of homotransplants of skin grafted between adult mammals other than monozygotic or identical twins, presumes an immune reaction which is either an anaphylactic response or a reaction of delayed cutaneous sensitivity The theory of the immune reaction presumes the development of circulating antibodies though investigations in immunology have failed to demonstrate them The delayed sensitivity reaction is associated with cells in which an antibody has not been detected The accumulated evidence of the past few years indicates that transplantation immunity probably belongs to this second category since it is similar to the tuberculin reaction and to sensitization reactions of the delayed type Although the precise immunological cause of destruction of homografts is controversial most workers have assumed the validity of the active immunity hypothesis Most of the experimental work involving homotransplantation has been along one of two lines of attack (1) those experiments which involve mitigation of the immune response i e the inhibition of the immune reaction in the host by physical or chemical means Techniques include the employment of irradiation antihistaminics, adrenocorticotropic hormone splenectomy cortisone skin extracts plasma and serum (2) The experiments in which attempts are made to alter the graft to change its specificity or antigenic properties thereby making it more acceptable to its new host Although the latter experiments are fewer in number, such physical methods as freeze dry exposure to high temperatures and preservation of homotransplants in a mixture of serum and balanced salt solution with antibiotics at 0 to 8°C have been employed to alter homografts before transplantation Some workers have attempted to elicit desensitization of homografts by growing small specimens *in vitro* with serum of the recipient added in increasing amounts to the medium during the culture period Stone Owings and Gey[8 9] and also Gaillard[4 5] obtained growth of stroma and encapsulation of the human thyroid and parathyroid *in vitro* Subsequent grafting of the endocrine tissue into patients afflicted with tetany relieved the condition More recently Blocker Pomerat and Lewis[1] also Earle and Evans[3] worked with human skin and obtained growth of epithelial cells *in vitro* Subsequent transfer of pure culture of sheets of epithelial cells to severely burned humans has been limited and has met with varying results This report is the presentation of data and observations made during the past 1½ years on the alteration of free grafts of skin (whole thickness) by their preservation *in vitro*, prior to homotransplantation in media containing homogenous embryonic extract in low concentration Experiments were carried out in animals (mice) and in human volunteers Human embryonic extract was not available for the experiments in men

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Preservation and Transplantation Technique (Mouse) Grafts are excised from the depilated skin of the dorsum of the anesthetized adult mouse using aseptic technique. Grafts varying from 4 to 8 mm are taken with the von Graefe knife and transferred to a sterile Petri dish containing balanced salt solution (Gey's solution or Hank's solution). Connective tissue and subcutaneous fat is removed from the grafts by dissection. They are washed several times in balanced salt solution and transferred to a mica coverslip in a drop of homologous unheparinized plasma. One part of homologous or heterologous (chick) embryo extract is added and clotting is allowed to proceed for 10 min at 37 C. The mica coverslip is placed in a Pyrex test tube to which is added the maintenance media consisting of Hank's balanced salt solution 10 to 15 parts, phenol red as indicator, homologous serum 2 to 3 parts, homologous placental extract 1 to 2 parts, human cord serum 1 to 2 parts, human carcinoma ascitic fluid 1 to 3 parts, and dilute homologous embryo extract (1 100 or 1 500) 2 parts, to make a composite of 20 parts. The test tubes are placed in a roller drum and maintained at 10 revolutions per hour as designated by Gey and Gey.[6] Occasionally Carrel D 3.5 mm flasks were employed instead of the Pyrex roller tubes. Within 24 hrs the coagulum at the periphery of the explant is liquified and slow growth is noted. Periodic explantation for 24 hrs to standard tissue culture media with high concentration of embryo extract provides a check of viability. The maintenance media is completely replaced every 72 hrs and if the pH becomes too acid the specimen is transferred. It is possible to maintain the grafts under these conditions for periods of 3 to 19 weeks prior to transplantation to a new host. Seventy eight adult mice of both sexes, different strains and different colors of coat (Swiss albino and C_3Br) have been used in the experiments reported herein. This selection ensured maximum genetic diversity. Preparation of the host and the site for the graft in the mouse was similar to that reported previously from this laboratory. After the host was anesthetized the hair was removed by a depilatory cream and the dorsal fold of the skin was immobilized in a modified Joslin traction splint.[7] Modifications included reduction of the arch of the splint to diminish the tension exerted on the soft tissues of the dorsum and the addition of 2 types of lateral outriggers to aid in maintaining the dorsal fold in an upright position so that angulation of the blood vessels did not occur. In addition, multiple grafts were applied to a single host. This afforded a means of comparing microscopically the reaction of a single host to a fresh autograft, to a homograft and to a heterograft, each of which had been maintained *in vitro* for prolonged periods prior to transplantation. This technique diminished the need for excessive numbers of animals since each animal served as its own control.

Preservation and Transplantation Technique (Human) The technique of preservation of human skin was similar to that employed with experimental animals. Specimens were excised and freed of subcutaneous tissue, washed in balanced salt solution and mounted in plasma clots in Kohl flasks. Maintenance media were the same as used with experimental animals except that human embryo extract was not available. Specimens of skin for these experiments were obtained in the excision of decorative tattoos from the forearm or chest. It was hoped that the tattooed specimens would offer

unquestionable proof of success in the event that one or more of these grafts should survive indefinitely. They were preserved in the culture media at 37 C for 1 to 3 weeks. One of the major problems in these experiments was created by the lack of homologous human embryo extract. In addition several specimens had to be discarded because of contamination by mold (actinomyces). Following preservation the explants were removed from the plasma clot, washed thoroughly in balanced salt solution and transferred to a thin dermal bed on the left forearm of the human volunteer. Grafts were sewed in place by interrupted sutures, the tails of which were tied over a bolus of cotton waste. The arm, forearm and hand of each volunteer were immobilized in a plaster cast.

Statistics and Observations. *Animal experiments* are reported in which 265 free, whole thickness skin grafts have been transplanted (Table 1). Of these 18 were fresh, untreated autografts. All of these healed and became indistinguishable from the surrounding skin. Seven autografts maintained *in vitro* 2 weeks before transfer back to the original donor were employed as controls. No deleterious effect was noted as a result of this technique of preservation. Of the 226 homografts maintained *in vitro* prior to transplantation 106 were intrastrain homografts, i.e., donor and recipient were of the same strain, and 120 were interstrain grafts, i.e., donor and recipient were of different strains. In addition, 14 heterologous grafts were transplanted. These were not heterografts in the true sense since they were of embryonic skin of the chick, not yet as differentiated as mature skin. In order to ascertain whether there was any reaction toward the maintenance media 5 plasma clots from which the transplants had been removed were placed in the recipient sites. Except for moderate increase in the vascularity of the recipient bed nothing of importance was noted. All of these grafts were maintained *in vitro* for a minimum of 1 week and the majority for 1 to 3 weeks. Several grafts were preserved for more than 15 weeks. There was definite prolongation of the survival period of intrastrain homografts as a consequence of maintenance *in vitro*. Untreated fresh homografts usually survive less than 10 days. An increasing number of days of preservation of the grafts (up to 4 weeks) was followed by increased number of days of survival of the homografts. Periods of survival of grafts preserved 4 to 9 weeks prior to transplantation gave varying results. There appeared to be correlation between increased time of maintenance *in vitro* and increased duration of survival on the

Table 1. Experiments on Transplantation of Preserved Skin Grafts

	NO. TRANSPLANTS
Autografts	18
Autografts preserved *in vitro*	7
Homografts preserved *in vitro*	
Intrastrain	106
Interstrain	120
Heterologous grafts preserved *in vitro*	14
Plasma clot controls	5
Total number animal experiments	270

Table 2 Intrastrain Preserved Homografts of Skin

No. days prior preservation	No. grafts	No. days survival on new host
7	27	12.9
14	17	2[illegible].9
21	10	15.2
28	[illegible]	17.6
35	1	11
42	6	8.8
49	[illegible]	11.1
56	1	18
63	5	17.4

Table 3 Interstrain Preserved Homografts of Skin

No. days prior preservation	No. grafts	No. days survival on new host
7	37	19.4
14	12	20.6
21	6	10.8
28	12	16.4
35	2	16
56	6	7.6
63	7	17.6
70	2	permanent takes
77	5	7.8
84	2	4
105	3	4
112	2	3
133	1	2.3

new host (Table 2). Similar data were obtained with interstrain homografts following preservation *in vitro*. There was an increased survival period compared with that of fresh homografts and also a positive correlation between increased survival time and increased periods of maintenance *in vitro* (Table 3). Although the experiments are too preliminary to warrant positive conclusions, this study indicates that preservation periods of 2 or 3 weeks are probably the most effective in the prolongation of survival of preserved homografts of skin. Significant decrease in viability was noted with grafts which had been maintained longer than 11 weeks. Many aspects of preservation remain to be investigated more fully, viz., temperature, composition of media and acidity.

These studies indicate that highly acid medium is not favorable to the prolongation of survival time of homotransplants of skin. In some cases where the proportion of embryo extract was increased, vigorous proliferation of hair was noted on the explant. This was associated with very rapid destruction of the graft following transplantation. In 2 cases, interstrain homografts, preserved as outlined above, have persisted for more than 12 months, apparently permanent successes. In 1 animal an interstrain homograft which was preserved for 2 weeks *in vitro* has survived for 14 months. It is assumed that some loss of specificity of the homograft occurs as a consequence of preservation *in vitro*. This is substantiated by

the interesting observation that multiple homografts maintained *in vitro* under different conditions though from a single donor elicited a wide variety of reactions from the same recipient These individualized reactions of a host toward multiple homografts from the same donor may indicate some loss of specificity as a consequence of preservation

Experiments in the human were undertaken in view of the results obtained with experimental animals Four human volunteers have been studied Prior to reception of the homografts which were preserved 2 to 3 weeks before transplantation several laboratory tests were done These included complete blood counts and electrophoretic studies These tests were repeated after homografting None of the preserved human homografts survived All were desquamated within 3 weeks But the violent reaction of the rejection of the homografts was not seen

We believe that the failure of preserved homografts to survive transplantation in the human was partly due to the lack of homologous embryo extract All grafts were preserved in media containing heterologous (chick) embryo extract This may account for the discrepancy of results between human and experimental animals It is planned to pursue these studies in the human

SUMMARY

Preliminary studies in animals indicate that the survival time of whole thickness homografts of skin preserved in tissue culture media is prolonged in comparison to that of untreated homotransplants The prolongation of survival time of homografts in experimental animals seems to be correlated with increased periods of preservation *in vitro* Homografts seem to undergo some loss of specificity as a consequence of preservation *in vitro* since grafts from the same donor which were maintained under varied conditions elicited quite different reactions from the host Clinical investigation on the effect of preservation of homografts of skin in tissue culture media prior to transplantation have been carried out in 4 human subjects who volunteered for the experiments All grafts failed to survive but the violent reaction of homograft rejection was not noted

REFERENCES

1 Blocker T G Pomerat C B and Lewis S R Research opportunities with the use of culture of living skin Plast & Reconstructr Surg *5* 283 1950

2 Conway H Stark R B Lazzarini A A and Sedar J D Observations on the development of circulation in skin grafts XII Effect of prolonged maintenance *in vitro* upon the survival of living autologous and homologous skin grafts in mice (preliminary report) Plast & Reconstructr Surg *15* 430 1955

3 Evans V J and Earle W R The use of perforated cellophane for the growth of cells in tissue culture J Nat Cancer Inst *8* 103 1947

4 Gaillard P J Growth differentiation and function of explants of some endocrine glands Growth Phila *2* 139 1948

5 Gaillard P J Transplantatie van gekweekte weefsels bij de mens Konink Nederl Akad Weten *58* 4 1949

6 Gey G O and Gey M K The maintenance of human normal cells and tumor cells in continuous culture I Preliminary report cultivation of mesoblastic tumors and normal tissue and notes on methods of cultivation Am J Cancer *27* 45 1936

7 Joslin D A tissue chamber and splint for the mouse Science *115* 601 1952

8 Stone H Owings J and Gey G O Transplantation of living grafts of thyroid and parathyroid glands Ann Surg *100* 613 1934

9 Stone H Owings J and Gey G O Living grafts of thyroid and parathyroid glands Surg Gyn Obst. *60* 390 1935

ENZYMATIC ACTIVITY OF BANKED SKIN*

James T Chamness Jack Kayes Falls B Hershey and
I A Traylor

Although clinical trials have shown that nonviable frozen and dried homografts are useful biological dressings for burns and denuded areas and can be stored conveniently, it has seemed desirable to maintain viability Current methods of evaluating viability require grafting and observation of large groups of animals so that a more rapid and simple method would be desirable

It seems certain that enzyme activity is essential for the survival of skin and that the best media and methods of storage rates and conditions of freezing and thawing will be those which preserve enzyme activity After Hershey and Mendle reported their quantitative assay of various enzymes in the different layers of skin[1] we searched for semiquantitative histochemical tests of enzyme activity which might be correlated with viability

Tetrazolium dyes have been used to test the viability of seeds and to localize the activity of respiratory enzymes in microscopic sections of various tissues[2] Hydrogen removed from various substrates by their specific enzymes combines with oxygen after a series of enzymatic reactions (Figure 1) Although the exact mechanism is not clear when flavoproteins or other enzymes add hydrogen to the colorless tetrazolium chloride (TTC) an insoluble red formazan pigment is deposited in the cell The amount of TTC reduced is an index of the enzyme activity (Fig 1)

When fresh split thickness skin grafts are incubated in 0 1 per cent 2 3 5 triphenyl tetrazolium chloride (TTC) in 0 125 M PO_4 buffer pH 7 4 for 60 minutes at 38° C under anaerobic conditions the epidermis turns a bright and uniform red even without added substrate Dermis is unstained except for hair follicles skin appendages and blood vessels (Fig 2) Mouse and rabbit skin show similar appearance and their subcutaneous muscle stains deeply also In the presence of oxygen however, color is deposited only in creases and folds Anaerobic incubation gives darker and more uniform color also with aerobic substrates such as succinic acid or lactic acid 0 025 M As in other tissues succinic dehydrogenase produced more color than developed without added substrate (endogenous metabolism) Samples were graded 1 to 4 plus according to their gross appearance

The Nadi reaction is a test for cytochrome oxidase which is the last enzyme of the hydrogen carrier system[4] (Fig 1) The skin grafts are incubated aerobically with a fresh mixture of equal parts of 0 18 per cent alpha napthol in 95 per cent ethanol and 0 216 per cent aqueous solution of dimethyl para phenylene diamine hydrochloride This is buffered to pH

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*From the Department of Surgery Washington University School of Medicine the St Louis City Hospital and the Surgical Service of the Veterans Administration Hospital, St Louis Missouri Aided by grants from the Harry Freund Memorial Foundation the St Louis City Hospital Research Institute and the U S Public Health Service (RG-4102) to Dr Falls B Hershey The authors are very grateful for the timely technical assistance of Barry M Kayes and Jane Fleming Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director The statements and conclusions published by the authors are the result of their own studies and do not necessarily reflect the opinions or policy of the V A

SUBSTRATE AH_2 | OXIDIZED DPN or TPN | REDUCED CYTOCHROME c, b, a or FORMAZAN (RED) | $\frac{1}{2}O_2$

OXIDIZED SUBSTRATE A | REDUCED DPN H_2 or TPN H_2 | OXIDIZED CYTOCHROME or TTC (COLORLESS) | H_2O

DEHYDROGENASE | FLAVOPROTEIN ENZYMES | CYTOCHROME OXIDASE

NADI REAGENTS + $\frac{1}{2}O_2$ → INDOPHENOL BLUE

Fig 1 Schematic representation of the hydrogen transport system

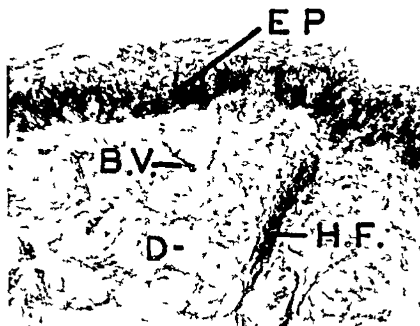


Fig 2 Human skin X100 Tetrazolium dye reduced by endogenous dehydrogenases

EP—Epidermis
D—Dermis
H F—Hair Follicle
B V—Blood Vessel

Note that dye is deposited in epidermis and skin appendages

7 2 to 7 4 with 0 3 per cent sodium carbonate or 0 125 M phosphate Cytochrome oxidase of the epidermis forms large deposits of the water insoluble dye indophenol blue after 20 to 30 minutes at 20 C This reaction is blocked by cyanide or the absence of oxygen Although spontaneous auto-oxidation turns the solutions blue passive staining is minimal

Split thickness grafts were taken postmortem during 37 autopsies No antiseptics or vaseline were used Small pieces were smoothed and stapled

on to stiff nylon mesh and tested while fresh. The remainder were rolled in moist saline gauze, wrapped in aluminum foil and stored at 4° C in a refrigerator until used.

Fresh skin showed strong succinic dehydrogenase activity and cytochrome oxidase activity. These enzymes were most stable with storage. Skin from patients over 12 to 24 hours postmortem usually showed less endogenous dehydrogenase activity and these enzymes were least stable with storage or with freezing. Skin from a patient treated with nitrogen mustard showed loss of succinic dehydrogenase activity after only 8 days storage. Fresh skin from this same patient had only slight activity of the anaerobic endogenous dehydrogenases (TTC reduction) and none after 1 day storage. Fresh skin with strong endogenous activity has maintained it during 2 to 3 weeks storage and occasionally thereafter enzyme activity has been enhanced by addition of glucose. This suggests that substrate reserves have been depleted during storage and that the media should be supplemented with glucose. With longer storage separation of epidermis and dermis was frequently noted and enzymes were completely inactive.

Frozen and dried skin has many active dehydrogenases[1] but evidently the subsequent enzymes in hydrogen transport are damaged by drying since no TTC reduction or indophenol blue formation have been demonstrated in frozen and dried skin thus far.

Effects of various conditions of freezing and thawing on the survival of skin grafts on rabbits have been studied by Billingham and Medawar.[5] They reported that pretreatment with 15 per cent glycerin to prevent ice crystal formation was helpful and that skin frozen slowly and thawed quickly was more likely to be viable. For clinical use we froze our postmortem homografts slowly after 60 minutes pretreatment in Ringer's lactate with 15 per cent glycerine, thawed them quickly in normal saline at 37°C. Although useful as a biological dressing[6] for 10 to 14 days they lasted no longer than nonviable frozen and dried skin on 7 cases. We doubt the viability of such grafts and therefore have tested the effects of various freezing methods on the activity of skin enzymes.

Fast freezing to −185°C was done by immersion in liquid nitrogen for 1 to 5 minutes; other grafts were frozen slowly during 30 to 60 minutes by placing them in a double walled vessel immersed in dry ice and acetone (−85°C) and then transferring them to liquid nitrogen (−185°C). Grafts were thawed rapidly by immersion in Kreb's mammalian Ringer's solution at 37°C for a few minutes or thawed slowly in a beaker surrounded by chipped ice. Succinic dehydrogenase was least damaged by these manipulations but freezing and thawing usually caused considerable loss of endogenous TTC reduction unless the grafts had pretreatment with 15 per cent glycerine in Ringer's lactate or Kreb's solution for 1 hour. However the glycerin did not always protect enzyme activity completely. No conclusions can be drawn regarding the respective merits of fast or slow freezing or thawing since the results are not consistent. Moisture content, thickness of the grafts, duration of storage prior to freezing and other variables may influence the freezing process—and in some instances it appears that selective permeability is impaired and some enzymes or endogenous substrates may leak out during incubation. Unfrozen controls however have always shown uniform activity.

Various methods of sterilization of tissue have been used Mouse grafts were sterilized by incubation in 1 per cent beta propriolactone (BPL) for 2 hours at 37°C during which the pH was adjusted to 7 1 by titration with 10 per cent sodium carbonate[7] These grafts showed inactivation of succinic dehydrogenase and cytochrome oxidase Eight such grafts all sloughed 10 days after grafting on to mice whereas 7 control grafts grew and only 1 sloughed By courtesy of Dr John Trump and Kenneth A Wright of the Massachusetts Institute of Technology human grafts were exposed to 0 5 to 10 000 000 rep at 4°C and at room temperature using their van der Graaf accelerator There was a gradual decrease of Nadi reaction and endogenous TTC reduction with doses over 500 000 rep Succinic dehydrogenase however was only partially inactivated even by 10 000 000 rep Further work is in progress

Survival of human skin on mice zoografts has been reported[6] but in our hands the take or survival of such zoografts is too variable to be useful

CONCLUSIONS

1 The epidermis of human skin showed marked succinic dehydrogenase and endogenous dehydrogenase activity as measured by reduction of tetra zolium dye

2 The epidermis of human skin showed marked cytochrome oxidase activity as measured by formation of indophenol blue (Nadi reaction)

3 After storage of human split thickness grafts anaerobically at 4°C in moist saline gauze for several weeks TTC is not reduced by the dehydrogenase until glucose is supplied After longer storage all enzyme activity is lost and cannot be restored

4 Freezing and thawing caused considerable loss of endogenous dehydrogenase activity unless skin was pre treated with glycerin However, glycerin did not always protect enzyme activity completely

5 Sterilization of mouse skin with beta propiolactone destroyed dehydrogenase and cytochrome oxidase activity and this skin was nonviable

6 If future work shows similar correlation of viability and tetrazolium reduction in human skin these methods will be rapid and convenient ways of testing the viability of banked skin prior to use and in evaluating new methods of skin preservation

REFERENCES

1 Hershey F B and Mendle B J Quantitative histochemistry of burned and normal skin in Surg Forum 1953 Philadelphia W B Saunders Co 1954 p 745

2 Antopol W Glaubach S and Goldman L Pub Health Rep 63 1231 1238 1948

3 Black M M., Zweifach B W and Speer F D Tetrazolium salts A new tool in general and experimental pathology Am J Clin Path 23 332 1953

4 Dye J A Improved colorimetric method for determining quantitatively the indo phenol oxidase content of animal tissues Proc. Soc. Exp Biol N Y 24 640 1926 27

5 Billingham R E., and Medawar P B The freezing drying and storage of mammalian skin J Exper Biol., Lond 29 454 1952

6 Brown J B., Fryer M P., Randall P., and Lu M., Postmortem homografts as "biological dressings" for extensive burns and denuded areas Ann Surg 138 618 1953

7 Trafas P C., Carlson R E., LoGrippo G A., and Lam C. R Chemical sterilization of arterial homografts A M A Arch Surg 69 415-427 1954

LABORATORY INVESTIGATIONS OF SKIN VIABILITY FOLLOWING PRESERVATION BY VARIOUS METHODS*

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AND LUCY KING

Homografts are generally recognized as life saving by the temporary coverage they afford to the patient with extensive skin loss. Homografts from *postmortem sources* provide biological coverage of open areas, are preserved by various methods and are being stored in a *skin bank* for use at any time.[1, 2, 3, 4, 5]

The mouse (C 57 Leiden) has been used as an experimental animal and has proven to be a valuable means for viability studies. Preservation and storage methods, temperature and nutrient influences are being studied. Use of experimental animals is encouraged to avoid needless human trials.

Postmortem homografts transferred immediately and those stored at ice box temperatures take on a raw surface on the mouse not unlike autografts though survival is only temporary. Frozen or dessicated homografts

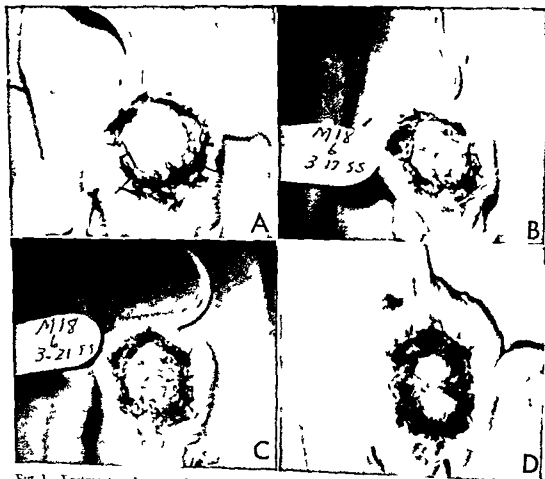


Fig 1. Postmortem homografts previously preserved at +4 C in nutrient Earle's solution and antibiotics for 11 months. (A) Appearance 7 days after transplant. (B) 13 days. (C) 17 days and (D) at 3 weeks. Function of homograft persisting over prolonged period.

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prepared by any combination of methods, may supply satisfactory coverage for a few weeks but they may only survive not actually take However by surviving a few weeks they may accomplish the aim of homografts even though they act only as a protective covering

Storage of Skin at +4°C. This is the simplest method of skin banking requiring a minimum of time and materials Ordinary refrigerator temperatures are generally available Fresh postmortem homografts are folded on saline antibiotic gauze sponges and kept moist in a jar until they are required They are ready for immediate use on the recipient They remain viable on the mouse for approximately one month as shown in Figure 1 If not used these grafts have been processed and stored at low temperatures or lyophilized

Nutrient Media has been added to the saline antibiotic solution and the survival time of postmortem homografts stored at +4°C studied 10 per cent serum has been added to buffered normal saline with 50 units of penicillin and streptomycin per cc Lowering of the pH by cell break down is shown by color change when phenol red is used Usually the media requires change about every 8 weeks or whenever it is cloudy or found contaminated

Low Temperature Skin Storage In the few clinical reports of the use of skin stored at low temperatures there seems to be considerable confusion

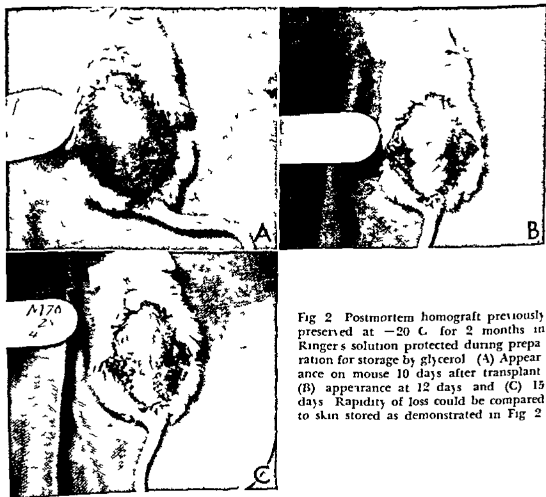


Fig 2 Postmortem homograft previously preserved at −20 C for 2 months in Ringer s solution protected during preparation for storage by glycerol (A) Appearance on mouse 10 days after transplant (B) appearance at 12 days and (C) 15 days Rapidity of loss could be compared to skin stored as demonstrated in Fig 2

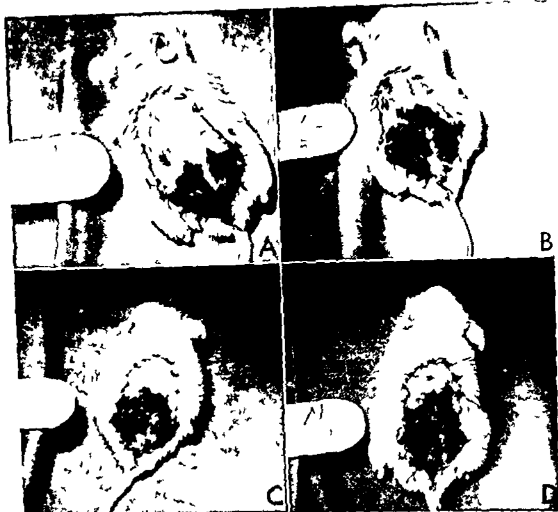


Fig 3 Postmortem homograft previously stored at −80° C for 2 weeks then used to cover denuded area on mouse (A) Appearance 5 days after transplant (B) at 8 days (C) at 12 days and (D) at 2 weeks Early loss of epithelium and rather rapid dissolution of derma demonstrated

in deciding whether the graft is viable or not However, there are many reports intimating that skin grafts do remain viable for long periods of time

Glycerol has been used because of its protective action on grafts in low temperature banking The skin is pretreated with 15 per cent glycerol in Earle s or Ringer s solutions for 1½ hours then blotted dry and placed in a glass jar Slow lowering of the temperature is done by placing this jar in another glass container in a dry ice alcohol mixture when storage of the inner jar is to be at −80 C To thaw the graft is directly or in its container placed in sterile Ringer s solution at 37 C The glycerol is removed by soaking the graft in Ringer s or Earle s solution for 1 hour at room temperature

Using this method of glycerol protection split thickness skin grafts have been stored at −20 C −40 C and −80°C

Low Temperature Storage without Glycerol is also being investigated

Lyophilization Freeze drying results when skin is frozen and the ice is evaporated to a low temperature condensor without melting Some cells

in a skin graft may survive this process This is being investigated with lyophilized autografts and inbred animals in which homografts do survive Living cells have not been demonstrated by tissue culture in lyophilized skin which has been dehydrated to less than 1 per cent total water content After moistening lyophilized skin looks grossly like fresh skin Prolonged storage at room temperature in the glass containers is ideal

Lyophilization Technique is essentially that reported by Creech DeBakey and Cooley Changes in minor details are made and are reported frequently An efficient lyophilization apparatus is made by the American Instrument Company which completes the drying in less than 8 hours and has outlets for 25 tubes

Observations made of human postmortem grafts prepared and stored as previously outlined for the indicated periods of time then transplanted to the mouse are shown in Figure 3 These would seem to indicate that storage at −80°C does not prolong the survival of human skin long enough to warrant the difficulties of this type of storage as indicated in the comparative chart shown in Table 1 The −80°C temperature was maintained by keeping the jar containing the skin in an alcohol dry ice slush mixture All skin used on these mice was removed within 8 hours after death of the donor Comparison of those stored in nutrient at the various temperatures can be made This additive seems to prolong the survival on the mouse very little The appearance and general cleanliness of the wound during loss of the homograft is important as far as the clinical use of homografts is concerned Cross contamination from a homograft postpones the time permanent healing with autografts can be done Immediate

Table 1 Postmortem Homografts Stored at Various Temperatures Then Transplanted to Mouse for Skin Survival Study

METHOD OF PRESERVATION AND STORAGE	NUMBER OF MICE	LENGTH OF STORAGE	NUMBER OF DAYS SURVIVED ON MOUSE	RATE OF CHANGE FROM SURVIVAL TO COMPLETE LOSS
+1 C Ringer's or Earle's solution and antibiotics	23	1 7 mo	17 7	rapid
		immediate to 1 mo	7 29	
+1 C with nutrient	32	6 5 mo	17 7	slow
		1 13 mo	7 27	
−20 C Ringer's or Earle's solution and antibiotics	18	1 5 mo	16 3	slow
		2 6 mo	10 20	
−20 C with nutrient	13	1 5 mo	15 7	slow
		2 5½ mo	10 21	
−80 C Ringer's or Earle's solution and antibiotics	2 ,	2 8 mo	13 9	irregular
		9 days 1½ mo	7 21	
−80°C with nutrient	13	3 2 mo	16 3	slow
		1 wk 1¼ mo	14 21	

coverage with the patient's own skin following loss of a fresh postmortem homograft has been done repeatedly. Sometimes the way homografts which have been stored at low temperatures are eventually rejected by the experimental animal makes immediate coverage with autografts impossible. This has been noted in particular in the experimental use of lyophilized skin.

SUMMARY

Postmortem homografts prepared and stored under various conditions are being studied using the mouse as an experimental animal. Time of survival and importance of homograft "take" are noted and compared.

REFERENCES

1. Brown, J. B., Fryer, M. P., Randall, P., and Lu, M.: Postmortem homografts as "biological dressings" for extensive burns and denuded areas. Ann. Surg., 138: 618-629, 1953.
2. Brown, J. B., Fryer, M. P., and Zaydon, T. J.: Skin bank for postmortem homografts. Surg. Gyn. Obst., 101: 401-412, 1955.
3. Brown, J. B., and Fryer, M. P.: Postmortem homografts to reduce mortality in extensive burns. J. Am. M. Ass., 156: 1163-1166, 1954.
4. Brown, J. B., Fryer, M. P., and Zaydon, T. J.: Establishing a skin bank. Use and various methods of preservation of postmortem homografts. Plastic & Reconstr. Surg. (In press).
5. Brown, J. B.: Homografting of skin: with report of success in identical twins. Surgery, 1: 558-563, 1937.

THE EFFECTIVENESS OF STERILIZATION OF CANINE COSTAL CARTILAGE BY COBALT^{60} IRRADIATION AND ITS FATE WHEN USED IN HOMOGRAFTS*

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The usual method of sterilization, preservation, and storage of homologous cartilage grafts at present is that devised by O'Connor and Pierce.[1] This method, which provides an effective means of preserving cartilage, entails the use of an aqueous solution of merthiolate for a period of 1 to 4 weeks before sterility is assured. The cartilage is then stored at 4°C. in the merthiolate solution until used. Although this method is effective, a simpler and quicker method of sterilization of costal cartilage by Cobalt^{60} gamma irradiation is presented here.

Brownell *et al.*[2] showed that intense irradiation with 3,000,000 rep will destroy most organisms including the viruses. The time required to administer 3,000,000 rep to a substance close to the Cobalt^{60} source is about 15 hours. Sterilization can be effected by this method in any substance including homologous blood vessels and bone grafts.[3, 4]

Preserved cartilage to be used as a graft is different from bone in that the former is a permanent graft while the latter is replaced or modified by host tissue. Therefore, the fate of *irradiated* cartilage in the host over a prolonged period of time becomes a consideration of great importance.

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METHOD

To evaluate the irradiation method of sterilization in comparison with that of O Connor and Pierce and at the same time to determine the easiest and most effective means of storage 4 groups of canine costal cartilages were prepared from young adult dogs The cartilage was divided into 4 groups

1 Cartilage sterilized and stored in merthiolate by the method of O Connor and Pierce as a control

2 Cartilage lyophilized by the freeze dry method in vacuum sealed containers and irradiated with 3 000 000 rep then stored at room temperature

3 Cartilage placed in sealed containers without vacuum irradiated with 3 000 000 rep and stored at room temperature

4 Cartilage placed in normal saline sealed in glass containers without vacuum irradiated with 3,000 000 rep and stored at room temperature

When removed from the donor animal all cartilages were purposely contaminated by dipping into broth containing mixed cultures of *Staphylococcus aureus, Streptococcus zymogenes, Escherichia coli Clostridium tetani, Clostridium botulinum* and *Clostridium sporogenes* Sterilization and storage were effected by the methods as outlined above It was noted that cartilage in merthiolate required at least 4 separate transfers before the first negative culture was obtained when spore forming organisms were used At the time the grafts were placed in dogs cultures were obtained from these cartilages Cartilage sections from each donor dog were sterilized by the 4 methods and placed in a single host dog Using sterile technique the cartilage was placed in 2 sites the rectus sheath and beneath the scalp on the pericranium The dogs were reoperated on at periods of 2 3 4 9 and 12 months for removal of the cartilages The incidence and degree of absorption were studied by comparative measurements of cartilage sections made before implantation and after removal and by microscopic study Storage time before implantation varied from a few weeks to 6 months

RESULTS

Seventy two pieces of cartilage were available for study Before implantation microscopic studies showed some loss of nuclei after irradiation Merthiolate treated cartilage had minimal nuclear change After storage for 6 months no further microscopic changes were noted in either group

Bacteriologic studies of all 4 groups showed sterilization of the bacteria previously used to contaminate the cartilage

The fate of the grafts was determined by 2 methods In the first the cartilage was carefully measured in millimeters prior to implantation and at the time of removal In the second method the cartilage was studied microscopically after removal The microscopic study generally showed correlation with the measured changes but in all the pieces the pathologist could find some evidence of cartilage absorption fibroblastic proliferation at the sites of absorption and occasional osseous transformation In some cartilage the absorption was great and in others very slight In the latter gross measurements and inspection showed no change Quantitative evaluation of absorption is difficult to obtain by microscopic study and therefore the results reported are based on the measured and gross changes

After a 2 months implantation of 8 pieces from the 4 groups of cartilage results were inconclusive because of the small number and the short implantation time We were unable to find some of the transplants The missing pieces in this group and all subsequent groups were classified as absorbed At 2 months absorption is improbable it is likely that infection and extrusion of the cartilage can account for the missing pieces

At 3 months examination of 8 pieces of cartilage showed slight decrease in size in 50 per cent the others measured the same Microscopically all showed some degree of absorption The merthiolate sterilized control cartilage showed more advanced changes than the irradiated cartilages at this time

At 4 months 16 pieces of cartilage were evaluated In the 2 dogs used a marked individual variation in the cartilage was noted The merthiolate control cartilage was the same as the cartilage irradiated in air and that which was lyophilized and irradiated All groups had 2 pieces intact with 2 pieces either absorbed or undergoing absorption The group irradiated in saline showed no change

At 6 months 13 pieces were studied from 2 host dogs The degree of absorption was greater The merthiolate cartilage samples were all absorbed or undergoing absorption The irradiated cartilage group had several pieces with no change and the remainder absorbed or being absorbed

At 9 months 16 pieces of cartilage from the same donor animal were studied in 2 different host dogs This again demonstrated the individual response to cartilage with 1 animal absorbing cartilage definitely faster than the other The merthiolate group had 50 per cent absorbing and

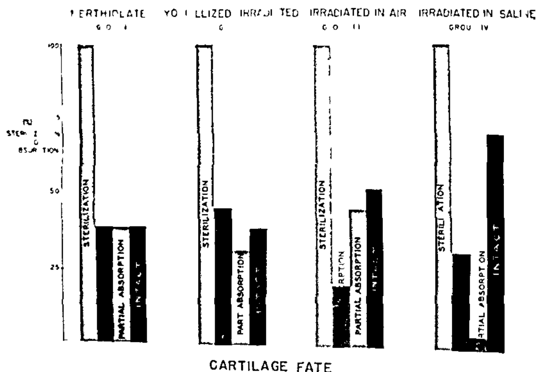


Fig 1

50 per cent intact, the lyophilized irradiated group had 75 per cent absorbing and 25 per cent intact the group irradiated in air had 25 per cent absorbing with 75 per cent intact those irradiated in saline had 100 per cent intact

At 12 months 11 pieces were removed from 2 dogs One merthiolate cartilage was still intact while among the 10 pieces of irradiated cartilage 3 were unchanged and 7 were absorbed or absorbing

In compiling composite figures for absorption rates the cartilage samples in all 4 groups were totaled with results divided into 3 categories The first group was cartilage absorbed the second partially absorbed and the third 'no absorption This included all the various intervals of time

1 The merthiolate group of 18 cartilages showed complete absorption of 33 3 per cent, 33 3 per cent were partially absorbed and 33 3 per cent were intact The average time of implantation was 5⅔ months

2 The lyophilized irradiated group of 18 cartilages showed complete absorption of 38 9 per cent, partial absorption of 27 7 per cent and no absorption in 33 3 per cent The average time of implantation was 7⅓ months

3 The group of 18 cartilages irradiated in air showed complete absorption of 16 7 per cent partial absorption of 38 8 per cent and no absorption of 44 4 per cent The average time of implantation was 5⅓ months

4 The group of 18 cartilages irradiated in saline showed 27 8 completely absorbed 5 5 per cent partially absorbed and 66 6 per cent intact The average time of implantation was 6⅔ months

The merthiolate group and the lyophilized irradiated group showed about the same results though the latter group had a longer implantation time Results in the irradiated in air group were slightly better but with slightly less time of implantation Results with the irradiated in saline group were definitely superior to those with all other groups

In studying the cartilage placed on the pericranium the 36 pieces showed 33 5 per cent absorbed completely 30 5 per cent partially absorbed and 36 per cent intact The 36 pieces of cartilage placed in the rectus sheath showed 25 per cent completely absorbed 19 5 per cent partially absorbed and 55 5 per cent intact The cartilage in the rectus sheath was not absorbed as rapidly as that placed on the pericranium

DISCUSSION

There was scattered absorption with no marked pattern except that in each host the tendency to absorb cartilage showed individual variation This scattered absorption was felt to be due to individual reaction of the host to hematoma and infection postoperatively at the cartilage implant sites or to trauma inflicted at the site postoperatively by the animal

The pliability of the cartilage was excellent for the stored merthiolate cartilage, and only slightly less so for cartilage irradiated and stored in saline Cartilage irradiated in air proved to be less workable After lyophilization and irradiation the reconstituting time was about 1 hour for canine cartilage and 1½ to 2 hours for control human costal cartilage and pliability was considered poor Lyophilization is a tedious process and the reconstitution time necessary is an undesirable feature at operation

SUMMARY

Using merthiolate treated cartilage as a control irradiated cartilage from 3 other groups was implanted in the rectus sheath and on the pericranium of the dog The cartilages were removed at varying intervals for study of degree of absorption in the various groups Of all cartilage groups the best results were found in cartilage irradiated in saline

Microscopic studies showed minimal change of cartilage after irradiation After storage for 6 months no further microscopic changes were noted

Rapid (15 hours) and adequate sterilization of cartilage can be effected by Cobalt 60 gamma irradiation using 3 000 000 rep

Irradiation of cartilage in saline in sealed tubes provides a convenient and satisfactory method for sterilization of cartilage The cartilage can be stored at room temperature in these containers without fear of damage to the cartilage

The results of this study indicate that homologous cartilage transplants which have been irradiated show a lesser degree of absorption than the merthiolate sterilized control cartilage

REFERENCES

1 O Connor G B and Pierce G W Refrigerated cartilage isographs Surg Gyn Obst *67* 796 798 1938
2 Brownell L E *et al* Progress report 2 (COO 90) Univ of Mich Eng Res Inst Proj M943 Ann Arbor Michigan January 1952
3 MaCris J A Sloan H and Orebaugh J E The use of cobalt as a sterilizing agent for aortic homografts I Effect of gamma ray irradiation upon the structural integrity of the graft in Surgical Forum 1953 Philadelphia W B Saunders Co 1954 pp 268 273
4 DeVries P H *et al* Univ Michigan M Bull *21* 29 February 1955

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pH AND SKIN GRAFTING*

RICHARD C YE AND SHERMAN F SAFFIER

It is well known that any area of the human body denuded of epithelial coverage following trauma or 3rd degree burn will rapidly become infiltrated by vascular buds and be transformed into a granulating surface Through this granulating surface a continuous and constant loss of body fluids occurs The exudate from any granulating area acts as an excellent culture medium for the bacteria therefore it would be essentially impossible to make any granulating surface sterile Thus a physiologic dressing namely a skin graft will be necessary to correct this condition The question however as to when the defect is ready to accept a skin graft can at times be quite perplexing Healthy granulation tissue ready to receive the graft presents a flat surface a bright or intense red color and a surface with a firm pebbly texture It is firm does not bleed easily has no odor and has practically no exudate except a small amount of fibrinous film (Fig 1A and 1B)

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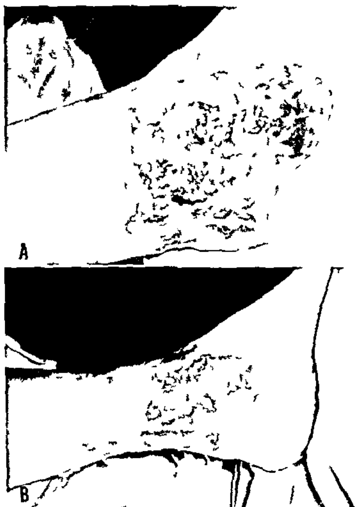


Fig 1 (A) Unhealthy granulation tissue covered with *exudate and necrotic tissue* pH 6 8 (B) 10 days later—granulation tissue became *healthier pH 7 4*

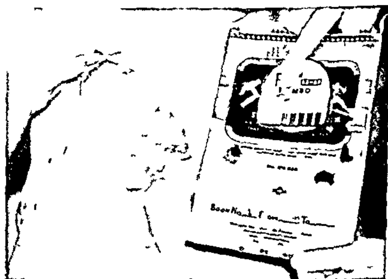


Fig 2 Method of measuring the pH of the granulation tissue

The above mentioned criteria are subject to many variations depending upon the surgeon s judgement This then is perhaps the main reason for referring the surface coverage problem to the plastic surgeons who have familiarized themselves with this problem by constant and frequent experience A less empirical and more scientific means for judging the

readiness of granulation tissue is obviously needed to save us from the wasted attempt of doing an unsuccessful skin grafting procedure

Farmers have measured the pH of the soil to predetermine the quality of their crop Perhaps a parallelism exists between the chemical state of soil and granulation tissue An experiment measuring the pH of the granulation tissues both on the ward and in the operating room was undertaken and the take of skin grafts correlated with the acidity present This simple procedure requires only a minimum of time and equipment Hydrion paper manufactured by Micro essential Laboratory Brooklyn New York with the range of pH from 6 0 to 8 0 was used and served the purpose well

The determination was made by pressing a small strip of pH paper against an area of granulation tissue which must be free of gross blood By comparing the change of color of the pH paper to the standard a reading of the pH of the granulation tissue was obtained immediately (Fig 2) Forty six cases of granulation tissue of various appearances and ages were studied Bacterial cultures from all granulation tissues together with their sensitivity tests for the various antibiotics were also undertaken

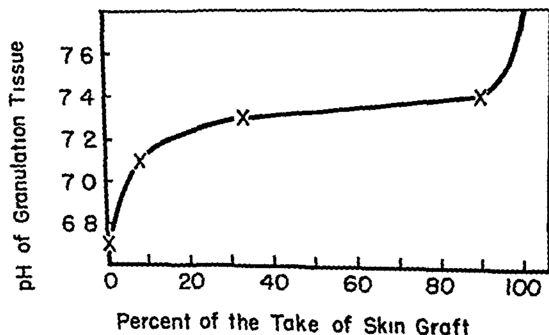


Fig 3 pH of granulation tissue and the take of skin graft

Table 1 pH of Granulation Tissue and Skin Graft

Number of cases studied	pH of granulation tissue	Average percent take of skin graft
6	6 8 7 0	0
10	7 0 7 2	8
7	7 2 7 4	33
10	7 4	90
16	Above 7 4	98 5

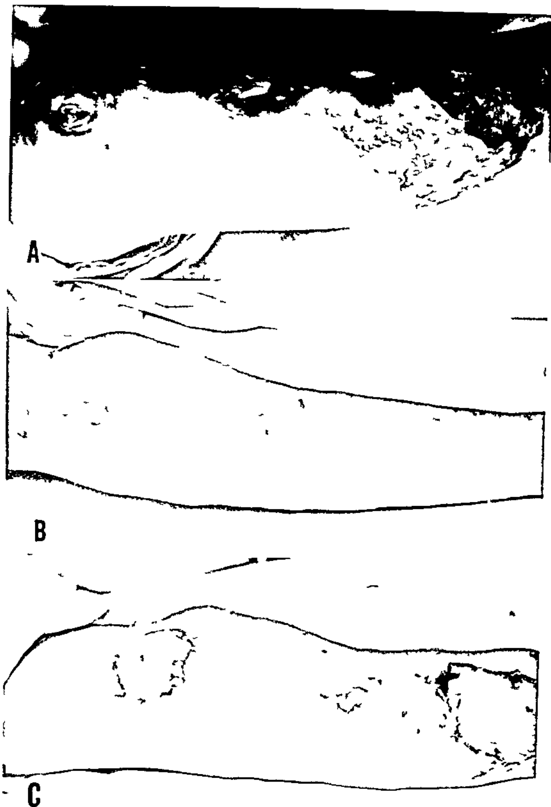


Fig 4 (A) Third degree burn of the leg covered with necrotic tissue and exudate pH 7 0 (B) Granulation tissue became healthier after debridement and daily normal saline dressings pH 7 4 7 6 (C) Same patient after skin grafting

The optimum pH of the granulation tissue as found by this study to accept the graft is over 7 4 The average of the take of the skin graft on granulation tissue correlated with pH was as follows pH 6 8 to 7 0 was 0 per cent take pH 7 to 7 2 was 8 per cent take pH 7 2 to 7 4 was 33 per cent take, pH 7 4 was 90 per cent take and above pH 7 4 the pH take was 98 5 per cent (Table I and Fig 3) No exact relationship between the nature of the bacterial culture and the pH of the granulation tissue could be determined however it was quite consistent that the granulation tissue covered with purulent material and thick exudate invariably gave a low pH reading

Photographs of an illustrative case are shown in Figure 1A B and C

DISCUSSION AND CONCLUSION

It is a well recognized fact that healthy granulation tissue in order to accept a skin graft should be in good physiological condition namely good blood supply, least amount of edema and a minimum of bacterial concentration Our clinical experiments measuring the pH of all granulation tissues have informed us that there is a definite correlation between the condition of granulation tissue and its pH the healthiest granulation tissue usually presenting a pH above 7 4 Whether this pH change is due to bacterial infection to the amount of exudate or to the blood supply, remains unknown It is our belief that this very simple clinical procedure may be of help to physicians who deal with skin grafting procedures We hope further investigative work will be done on this subject especially relating to the factor or factors which govern the change of the pH of granulation tissues so that the skin grafting procedure can be simplified and that the percentage of the take of the skin graft can be more certainly predicted

REFERENCES

1 Womack N On burns Springfield Ill Chas C Thomas pp 72 74
2 Meyer K and Kammerling E pH studies of malignant tissues in human beings *Cancer Res* *8* 513 518 1948
3 Voegtlin E and Kahler H The estimation of H ion concentration of the tissues in living animals Science *75* 362 364 1932
4 Voegtlin E DeEds F Kahler H Sanford M and Rosenthal S Simultaneous measurement in living tissues of electron equilibrium and hydrogen ion equilibrium Abstr of Communication of the 13th International Physiological Cong Am J Physiol *90* 546 1929

Urology

INTRODUCTION

SAMUEL A VEST

One of the problems following diversion of the urinary stream has been renal damage and electrolyte imbalance in the form of a hyperchloremic acidosis Three papers in the Forum this year by Drs Irvine Perry and Murphy and their associates have clarified some of the features of this problem With transplantation of the ureters to similar locations but employing different experimental procedures they reached a uniform conclusion with less renal damage due to ascending infection and less hyperchloremic acidosis occurs when the ureter is transplanted to a portion of the intestinal tract which has been separated from the fecal stream According to the compiled data in these papers the maximum preservation of renal tissue and minimum resorptive phenomena occur when there is a free flow bowel conduit uncontaminated by fecal contents and also when the extent of bowel exposed to urine is small in area

The mechanism of the acidosis following uretero intestinal anastomosis has been thought to be either a selective reabsorption of the chloride ion across the intestinal mucosa or else some selective renal tubular dysfunction or a combination of both The authors interpretations as to the cause of the hyperchloremic acidosis are somewhat divergent Irvine and his associates emphasized pathological studies of the kidneys after ureteral transplantation to various sites and found that pyelonephritis and acidosis were more pronounced when the ureters were transplanted to the intact colon but at the same time they noted no substantial anatomical change in the renal cortex or evidence of tubular damage to account for the greater incidence of hyperchloremic acidosis when pyelonephritis was present This group contended that enough normal renal tissue survived so that tubular damage was probably not a factor Furthermore a determination of the renal carbonic anhydrase activity as a measure of possible tubular dysfunction showed no difference in kidneys with and without hyperchloremic acidosis They concluded therefore that absorption of ions across the mucosa of the bowel rather than a renal factor was most likely the basic mechanism On the other hand Perry and his associates studied renal function in patients with similar types of urinary diversion by the careful measurement of glomerular filtration rates renal blood flow and the tubular reabsorption of chloride Their data indicated that hyperchloremic acidosis only occurred when there was a marked reduction in glomerular filtration rates and increased tubular resorption of chloride Furthermore their findings show that there is some slight reduction in renal function in all cases of ureteral diversion including cutaneous ureterostomies The interpretation of this paper tends to relate acidosis to tubular and glomerular

dysfunction occurring as a result of ascending infection One particularly interesting observation was the fact that seemingly normal appearing intravenous pyelograms which have been considered indicative of good renal function did not necessarily correlate with renal clearances or renal blood flow The depression of renal function was shown to be present to account for hyperchloremic acidosis even though the clinical intravenous pyelogram appeared normal Murphy and his associates determined concentration of various electrolytes in the mesenteric as compared with peripheral blood after ureteral transplantation to the right colon isolated ileum isolated sigmoid and the intact sigmoid colon According to their data chloride was reabsorbed in slight amounts equally in the ileum right colon and intact sigmoid colon but in no significant quantity by the isolated sigmoid conduit Urea was absorbed by all significantly less by the isolated sigmoid They interpreted these findings to indicate that the reabsorption of urea leading to an elevated urea nitrogen especially in the reservoir (intact sigmoid) type of transplant to account for the hyperchloremic acidosis They postulated that the elevated urea was one indication of urinary stasis or back pressure which might have some effect on renal function resulting in acidosis Boyce and Vest contended some years ago that reabsorption of (urea) ammonia conjugated with the chloride ion to be the major factor in the acidosis

Lapides made an extraordinary observation on bladder physiology when he demonstrated that essentially normal micturition is still possible with complete paralysis of the external sphincter (striated) muscle The fact that an individual can initiate and inhibit micturition entirely by the smooth muscle of the bladder and vesical neck is startling although Hugh Young noted years ago that patients could retain urine and void through an open perineal fistula which bypassed the external sphincter There are no known somatic nerves which innervate the bladder and no other known instances of voluntary control over smooth muscles mediated through the autonomic nerves If these experiments are valid then this example of voluntary *control over smooth muscle via the autonomic nerves leads to* speculation in this field as well as in that of psychosomatic medicine

Shoemaker s reconstruction of a urinary bladder using the serosal surface of a segment of ileum following total cystectomy in the dog is an example of highly imaginative surgery and may have considerable practical application Using a similar procedure he has successfully reconstructed the ureter which has not proved satisfactory by any prior experimental or practical methods Further work is needed to overcome or eliminate stricture formation at each end of the new graft

Both McCann and Morris and their associates have given us practical answers as to the fate of the kidney after complete interruption of the blood supply for various periods of time While their observations were limited to the kidney of the dog the results can be translated to some extent to the operating table of the clinician Morris has shown that clamping of the aorta above the renal vessels allows survival of the kidneys due to a minimal (20 mg hg) pressure in the aorta in which blood apparently reaches the kidney by some form of collateral circulation The anastomotic route through which vital circulation reaches the kidney deserves experimental study

The experiments of Miller and Vermeulen on the mechanisms controlling

solubility of calcium complexes in the urine is basic research in the general field of urolithiasis It is of interest that they found no difference in the urine of stone formers and normals in the amount of calcium in a form available for crystallization Such a finding throws less emphasis upon the role of colloids and their alleged effects on crystalloid solubilities

Goodwin has opened up an entirely new sphere of research with his study of the renal lymphatics a hitherto unexplored field The renal lymphatic system from recent evidence may be the site of beginning calcinosis wherein forms the necessary nidus for progressive crystallization or stone formation

Roger Baker has thrown further light upon renal physiology with his technique of removing and replacing the kidney in the dog so as to eliminate all autonomic nerve supply According to his data comprising various function tests there was no difference in renal function devoid of all autonomic nerves as compared to the normal Butcher has made further studies in physiology of the ureter and has shown that division and reanastomosis of the ureter has considerable effect upon the velocity of the peristaltic wave Interference with the conduction wave down the ureter (measured by electromyographic means) point to a physiologic rather than anatomic obstruction at the point of anastomosis to account for any hydronephrosis In some clinical cases however there is sometimes no pathologic dilatation above the point of anastomosis Apparently if contact and immediate healing occurs in the muscular wall of the ureter without interposition of fibrous tissue then the conduction wave is less seriously interrupted

AN EXPERIMENTAL COMPARISON OF RENAL DAMAGE AND ELECTROLYTE IMBALANCE FOLLOWING VARIOUS METHODS OF URINARY DIVERSION*

WILLIAM J. IRVINE, CHARLES McC. ALLAN AND DONALD R. WEBSTER

The frequency of renal damage and electrolyte imbalance following ureterocolostomy is well recognized. Ureteral transplantation to the isolated rectosigmoid[1] or the use of an ileal bladder[2, 3] appear to have reduced these hazards. However, long term studies of these exclusion methods are not available and the initial results obtained by ureterocolostomy alone are frequently satisfactory. An experimental study will be described contrasting the incidence of these late complications following diversion of urine to the colon in continuity, to the isolated rectosigmoid with proximal colostomy and to isolated segments of ileum.

The etiology of the hyperchloremic acidosis is disputed. Some authors[4] think selective reabsorption of chloride across the colon mucosa is the principle factor. Others[5-7] consider renal damage essential and suggest selective tubular damage might account for those cases of imbalance with normal pyelograms. Since tubular damage can produce hyperchloremic acidosis in certain nephrotic syndromes[8, 9] seen mainly in children, this explanation was not unreasonable. Tubular change in acidotic ureterocolostomy patients is seen usually in grossly damaged kidneys, making it difficult to interpret its relationship to hyperchloremia. In this experiment when chemical imbalance developed a careful search was made for evidence of a renal factor. The degree of cortical damage has been assessed histologically and the tubules studied for any selective histological changes. However, it has been shown that inhibition of the renal carbonic anhydrase[10, 11] can produce hyperchloremic acidosis. Ascending infection might produce hyperchloremic acidosis by inhibiting this enzyme without producing any histological changes. Therefore enzyme studies were carried out to determine if the renal carbonic anhydrase activity has been reduced in these dogs with hyperchloremic acidosis.

METHOD

This report is based on 39 mongrel dogs of both sexes and weights from 20 to 60 pounds. Only dogs with normal intravenous pyelograms were used in this experiment and all animals were apparently healthy.

Before surgery the following blood chemistry studies were done: Plasma Na and K (internal standard flame photometer), plasma chloride (Hiller's modification of Sendroy's method), blood creatinine (technique of Folin and Wu), blood urea and nitrogen (Van Slyke), and the plasma CO combining power (Van Slyke's manometric technique).

The dogs were then assigned alternately to 1 of 3 groups for surgery. The colon was prepared by enemata and oral neomycin (4 gm.) for 2 days prior to operation. Under intravenous nembutal anesthesia the abdomen was opened and the urethra divided between ligatures. The fundus of the

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bladder was then excised and its base with the undisturbed ureterovesical orifices used for implantation as follows

Group I (11 dogs) An ellipse was removed from the sigmoid colon and the bladder base anastomosed to the opening in two layers

Group II (13 dogs) The upper sigmoid colon was divided and the proximal end brought out as a terminal colostomy The bladder base was anastomosed to the open distal end of colon

Group III (12 dogs) A loop of terminal ileum 6 to 8 inches long was isolated with intact blood supply and ileal continuity restored by end to end anastomosis The bladder base was then anastomosed to the proximal end of the ileal loop the distal end being brought out to form an ileostomy stoma

By using the bladder base for implantation it was hoped to exclude the factor of faulty ureteral implantation from the experiment On recovery from operation, the dogs were maintained on a farm, each group receiving the same mixed diet Each dog returned at intervals of 2 to 3 weeks for repetition of the blood chemistry studies Approximately 9 months after surgery, the animal had its final blood chemistry estimations A second intravenous pyelogram was then done for comparison with the normal preoperative films The presence or absence of ureteral reflux was also determined radiologically by introducing 12 per cent NaI into the colon or ileum until a pressure of 30 mm Hg was recorded and then taking an x ray of the lower abdomen and pelvis After sacrifice the urinary tract and that part of the alimentary tract exposed to urine were studied microscopically and macroscopically Renal carbonic anhydrase activity was estimated by Davenport s technique[12] using a Warburg manometer with flasks calibrated at 2 2 cc volume (temperature 5 C) Homogenized preparations from the perfused renal cortex were used

RESULTS

General Nutrition of Dogs in Postoperative Period The dogs in the group with implantation into the colon in continuity lost weight and their condition deteriorated as the experiment progressed Two dogs in this group required intravenous fluids for nerve acidosis The dogs with ileal or rectosigmoid bladders maintained their weight and general condition throughout the experiment

Biochemical Estimations From the preoperative blood chemistry estimations mean values and the normal range of plasma K Na chloride CO_2 combining power blood creatinine and blood urea nitrogen were determined

(a) *Alterations in blood chemistry after urinary deviation to the colon in continuity* All 11 dogs developed hyperchloremic acidosis This imbalance usually started within 6 weeks of surgery In all these dogs fluctuations occurred but the chloride and CO_2 combining power returned to normal only for brief periods The blood urea nitrogen remained just above the upper limits of normality throughout the postoperative period The blood creatinine plasma Na and K estimations remained normal Figure 1a shows the postoperative course of a typical dog of this group

(b) *Alterations in blood chemistry after urinary deviation to isolated rectosigmoid* In the 13 dogs in this group the blood chloride and CO_2 combining power remained for the most part within normal limits However occasional brief periods of temporary imbalance developed The blood urea nitrogen remained at the lower limits of normal throughout the

COMPARISON OF BIOCHEMICAL FINDINGS
IN TYPICAL DOGS OF GROUPS I, II, III

LEGEND — PLASMA CHLORIDES (mEq/L) ————
— BLOOD UREA NITROGEN (mg %) — — — —
— PLASMA CO_2 COMBINING POWER (vol per cent) —•—•—•

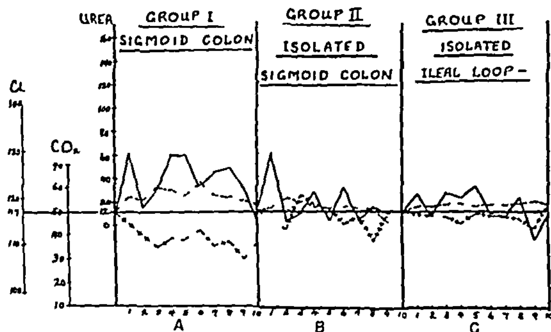


Fig 1

COMPARISON OF PLASMA CHLORIDE FOLLOWING
THREE METHODS OF URINARY DEVIATION
LEGEND TO INTACT COLON ————
ISOLATED SIGMOID COLON — — — —
ISOLATED ILEAL LOOP —•—•—•

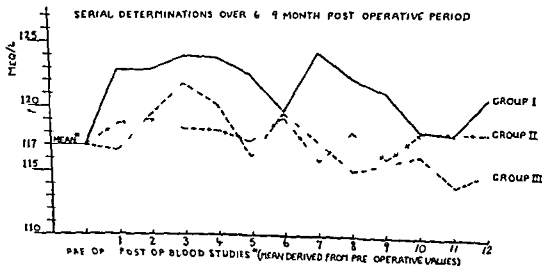


Fig 2

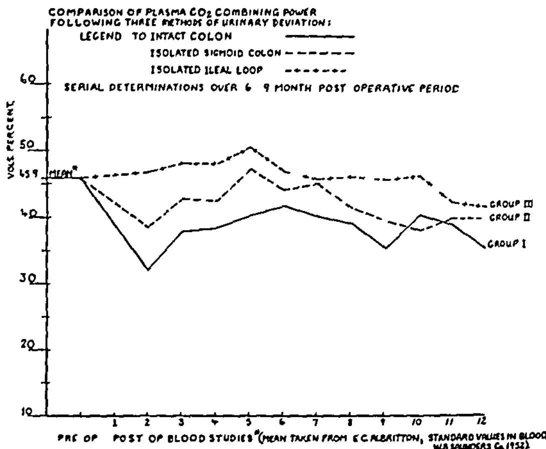


Fig 3

experiment The blood creatinine plasma Na and K estimations remained normal Figure 1b is typical of the postoperative course of dogs in this group

(c) *Alterations in blood chemistry after urinary deviation to isolated ileal loops* The plasma chloride CO_2 combining power and blood urea nitrogen remained within normal limits throughout the experiment and all other blood chemistry estimations remained normal Figure 1c demonstrates the total absence of fluctuations in the chloride, CO_2 combining power and blood urea nitrogen in dogs of this group

When the mean plasma chloride levels for each of the 3 groups is followed through the postoperative period the higher levels in Group I are apparent (Fig 2) Similarly when the means of the CO_2 combining power estimations are plotted for each group continued acidosis is limited to Group I (Fig 3)

Autopsy and Histological Studies (a) *Gross changes* The kidneys on section frequently showed some thickening of the renal pelvis in Group I and occasionally in the other groups The cortex frequently looked completely normal Sometimes tiny pin point foci of inflammatory change were seen in the cortex but these were never extensive No ureters were grossly dilated The implanted bladder was well healed The ureter mouths were all probed None were stenosed but some appeared patulous

(b) *Histological changes Kidneys* Pyelonephritis when present was graded to allow comparisons between the groups When inflammatory changes were almost limited to slight changes in the pelvis, the grade minimal was used When moderate a more marked pelvic reaction was present with extension of foci to the medulla and perhaps an occasional cortical

Table 1

GROUP	NO KIDNEYS	NO PYELITIS	MINIMAL PYELONEPHRITIS	MODERATE PYELONEPHRITIS	PRONOUNCED PYELONEPHRITIS
Group I	28	0	7	11	10
Group II	26	15	5	5	1
Group III	24	8	8	5	3

focus When the pelvic reaction was severe with extension of multiple foci into cortex pronounced pyelonephritis was present However when it was pronounced the multiple cortical foci were still small and the amount of normal cortical tissue not yet extensively reduced Table I gives the incidence and degree of pyelonephritis in the 3 groups of dogs (See Table 1)

Changes in the renal tubules The tubular changes were studied with the help of Dr D Waugh of the Pathological Institute Although only dogs in Group I developed chemical imbalance the tubules were essentially similar in all 3 groups and for the most part normal except when immediately adjacent to an inflammatory focus The extensive subnuclear vacuolation or subepithelial calcification seen in some nephrotic syndromes associated with hyperchloremic acidosis was not observed in these kidneys

The ureters Ureteritis when present usually paralleled the changes in the renal pelvis

Alimentary mucosa exposed to urine After 9 months exposure to urine the colon and ileal mucosae were essentially normal with no evidence of metaplasia to epithelium of transitional type

Comparison between Normal Preoperative Intravenous Pyelogram and Pyelogram before Sacrifice The changes seen were usually minor consisting of slight dilatation of the renal pelvis When this renal dilatation was accompanied by alteration in the pattern of the calyces it was described as moderate Concentration of the dye was reasonable in all cases No gross hydronephrosis was seen The results obtained in the different groups are given in Table 2 (See Table 2)

Radiological Evidence of Sodium Iodide Reflux up Ureters from the Intestine The incidence of reflux in each group is given in Table 2 Under the conditions of this experiment the ureterovesical valve allowed reflux in

Table 2

NO DOGS WITH SATISFACTORY STUDIES	NO PYELOGRAPHIC CHANGES	MINIMAL CHANGES UNILAT	MINIMAL CHANGES BILAT	MODERATE CHANGES UNILAT	MODERATE CHANGES BILAT	% WITH PYELOGRAPHIC CHANGES	% WITH URETERAL REFLUX
Group I							
11	3	3	2	2	1	72.7	63.6
Group II							
10	9	1	0	0	0	10	0
Group III							
11	11	0	0	0	0	0	0

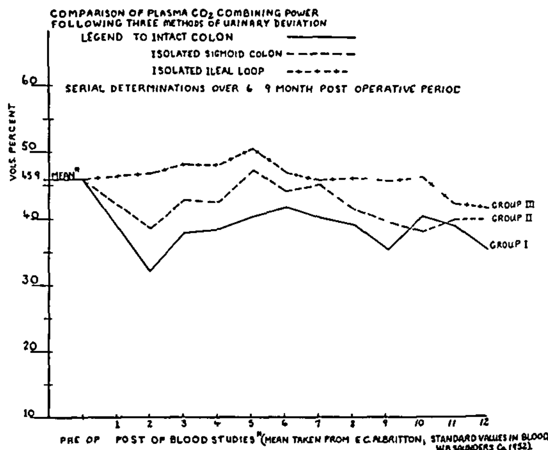


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Group I							
11	3	3	2	2	1	72.7	63.6
Group II							
10	9	1	0	0	0	10	0
Group III							
11	11	0	0	0	0	0	0

63 6 per cent of dogs in Group I No reflux was seen in the groups with fecal exclusion

Renal Carbonic Anhydrase Activity and Hyperchloremic Acidosis A concentration curve was obtained using kidneys of normal dogs A concentration suitable for assaying differences in enzyme activity was chosen from this curve and used in all estimations The range of activity was then estimated in normal dogs in 6 dogs of Group I with chemical imbalance and in 6 ileal bladder dogs with normal blood chemistries The range was closely similar in all 3 groups No reduction was observed in the dogs with hyperchloremic acidosis

COMMENT

1 **The Effect of Exclusion Techniques on Renal Damage** When the histological findings in Table 1 are analyzed statistically the incidence of normal kidneys is significantly higher in Groups II and III as compared to Group I The slight difference between Groups II and III is not significant When pyelonephritis is present the incidence of pronounced pyelonephritis is significantly less in the dogs with exclusion procedures Although the I V pyelograms showed only minor changes after 9 months, these are almost limited to Group I There was no stenosis of the lower ureters to account for these changes The ureteral reflux studies demonstrate the effect of the fecal stream on the normal ureterovesical valve It suggests that attempts to *produce a good valvular mechanism in ureteral transplantation may* be defeated in some cases when the fecal stream is not excluded From the histological and radiological evidence in this experiment it would appear that exclusion techniques provide some degree of protection to the kidney Thus there are significant differences between the groups only 9 months after surgery Longer survival periods might be expected to accentuate those differences

2 **Effect of Exclusion Techniques on Incidence of Hyperchloremic Acidosis** The biochemical results demonstrate the superiority of these exclusion methods over implantation into the intact colon which always produced chemical imbalance The ileal bladder dogs gave completely normal blood chemistry results throughout the experiment The dogs with isolated rectosigmoid bladders gave only occasional imbalance However in this group the colon was divided much more proximally than would be done in a human case since the dog retains urine for long periods and a capacious bladder was considered desirable A smaller rectosigmoid bladder with frequent voiding would reduce the absorptive surface and probably give results as good as those obtained with ileal bladders

3 **Etiology of Hyperchloremic Acidosis** The biochemical data in this experiment lend support to the belief that absorption of ions across the colon mucosa is the most important factor The onset of imbalance soon after surgery and the reduction in severity of the chemical upset achieved by reducing the area of colon exposed to urine both favor absorption as the cause No evidence could be found that renal damage was a factor Although the kidneys in Group I had usually more pronounced pyelonephritis at the time of sacrifice there was still no substantial reduction in the area of histologically normal cortex and no evidence of selective tubular damage Nor was any reduction in the renal carbonic anhydrase activity detected by the manometric method described

REFERENCES

1 Pyrah L N Uretero colic anastomosis Ann R Coll Surgeons England 14 169 197 1954
2 Bricker E M Butcher H and McAfee C A Late results of bladder substitution with isolated ileal segment Surg Gyn Obst 99 469-482 1954
3 Annis D Hunter W R and Wells C The use of an isolated length of ileum as a urinary channel Brit J Surg 42 290 301 1954
4 Ferris D C and Odel H M The electrolyte pattern of the blood after bilateral ureterosigmoidostomy J Am M Ass 142 634 640 1950
5 Rosenberg M L The physiology of hyperchloremic acidosis following ureterosigmoidostomy J Urol Balt 70 569 580 1953
6 Kekwick H Poully J W Riches E W and Semple R Renal failure following ureterocaecostomy Brit J Urol 23 112 122 1952
7 Jacobs A and Stirling W B The late results of ureterocolic anastomosis Brit J Urol., 24 259 304 1952
8 Peterman M C Congenital chronic pyelonephritis in infants with evidence of tubular damage with normal glomerular function Am J Dis Child 69 291 294 1954
9 Baines C H Barclay J A and Cook W T Nephrocalcinosis associated with hyperchloraemia and low plasma bicarbonate Q J Med Oxf 14 113 123 1945
10 Mann T and Keilin D Carbonic anhydrase inhibition by sulphonamide derivatives Nature Lond 146 164 1940
11 Brazeau P and Gilman A Effect of plasma CO tension on renal tubular reabsorption of bicarbonate Am J Physiol 175 33 38 1953
12 Davenport H W and Wilhelmi A E Renal carbonic anhydrase Proc Soc. Exp Biol N Y 48 53 56 1941

EVALUATION OF PLASMA ELECTROLYTES AND RENAL FUNCTION IN PATIENTS WITH SIGMOID URINARY POUCHES*

FRANK A PERRY MICHAEL R DEDDISH HENRY T RANDALL
AND KATHLEEN E ROBERTS

Hyperchloremic acidosis has been described as a manifestation of various types of renal disease[1 2 3 4] and more recently observed in patients with diversion of the urinary stream after cystectomy[5 6] In patients with uretero sigmoidostomy repeated episodes of pyelonephritis[7] are usually associated with the progression of the hyperchloremic syndrome which may be characterized by progressive anorexia nausea vomiting ileus dizziness fever and dehydration Associated laboratory findings include an elevated plasma chloride lowered bicarbonate and pH and at times hypokalemia[8] The blood urea nitrogen is usually elevated and these patients may show a severe anemia and alterations in glucose metabolism Demineralization of bones as a consequence of negative calcium balance may also be present[9] Inability to concentrate the urine results in a fixed specific gravity and excessive renal losses of water

*From the Departments of Surgery and Medicine of Memorial Center and the Andre and Bella Meyer Physiology Laboratories of the Division of Experimental Surgery Sloan Kettering Institute New York N Y This work was supported by a grant from the U S Public Health Service (CS 9236 C) We are indebted to Dr D Clarke Dr A Brunschwig Dr V K Pierce Dr H Barber and Dr W Daniels who provided the patients for this study We also wish to acknowledge the invaluable technical assistance of Serge Denecko Margaret Hood Frankie Lawson Rita Lipton and Jeanette Louzon

Various methods for transplantation of the ureters after cystectomy include skin ureterostomies ureterosigmoidostomy with intact anus wet colostomy, ileal and cecal bladders and more recently, the sigmoid urinary pouch[10, 11] as performed according to the technique of Deddish This study was undertaken in an attempt to assess renal function in patients with these various types of urinary diversion and to compare renal function in patients with and without hyperchloremic acidosis The results of this work suggest that renal function was somewhat reduced in all the patients studied who had diversion of the urinary stream In the patients who developed hyper chloremic acidosis however there was a marked impairment of renal function which was preceded by repeated episodes of pyelonephritis or anastomotic stricture

METHOD

Renal function and plasma electrolytes were measured in a total of 13 patients Five of these patients had hyperchloremic acidosis when studied and the remainder had no measurable electrolyte disturbance Three patients had their ureters anastomosed to an isolated sigmoid urinary pouch Two patients had an ileal bladder and 1 patient had bilateral cutaneous ureterostomies 1 patient had an intact urinary tract 3 patients had bilateral nephrostomies and 3 patients had their ureters transplanted to the intact colon The studies carried out on these patients consisted of measurements of glomerular filtration rate renal clearance of para amino hippurate and renal tubular reabsorption of chloride The plasma electrolytes which were measured included sodium potassium chloride, carbon dioxide and pH The glomerular filtration rate was measured by means of inulin In the patients with ureters transplanted to an intestinal segment the urine was collected by means of an indwelling multiholed catheter In this way the urine was continuously drained away from the intestinal segment and the time in which it was in contact with the mucosa was negligible Plasma and urine analyses were carried out according to standard methods to be described elsewhere Renal tubular reabsorption of chloride and water were calculated by subtracting the excreted moiety from that filtered The filtered quantity was calculated as the product of the plasma concentration and the glomerular filtration rate utilizing a Donnan correction factor of 1 05

Table 1

Chloride mM/L	Plasma Bicarbonate mM/L	pH	Urine: Glomerular Filtration Rate cc/min	Urine: Chloride Reabsorption mEq/L g f	Urine: PAH Clearance cc/min	Urine: Water Excretion % of Filtrate
1 R N ureters in intact colon						
116	16	7 26	13	114	92	31 3
2 B R Skin ureterostomies						
116	18 2	7 2	18	116	64	17 6

Table 2

Chloride mM/L	Plasma Bicarbonate mM/L	pH	Urine			
			Glomerular Filtration Rate cc/min	Chloride Reabsorption mEq/L g f	PAH Clearance cc/min	Water Excretion % of Filtrate
1 Patient Z Sigmoid Bladder						
105	28 8	738	64	103	396	12 7
2 Patient F Sigmoid Bladder						
108	24 8	736	56	105	373	12 7

RESULTS

Table 1 illustrates findings in 2 typical patients with hyperchloremic acidosis Both of these patients had had recurrent attacks of pyelonephritis and demonstrated glomerular filtration rates which were reduced below 20 per cent of normal Of some interest was the observation that the renal reabsorption of chloride in these patients was in excess of normal As shown here the reabsorption of chloride ranged from 114 to 116 mEq /l of glomerular filtrate In these patients urinary water losses were also large amounting to 17 to 35 per cent of that filtered Similar findings were noted in all the patients with hyperchloremic acidosis regardless of the method used for diverting the urinary stream

Table 2 shows the data on 2 patients whose ureters had been implanted into a sigmoid urinary pouch As shown here neither of these patients had hyperchloremic acidosis and glomerular filtration and renal clearance of para amino hippurate was normal for their age Neither of these patients had shown any clinical or laboratory evidence of electrolyte imbalance or pyelonephritis during the interval following their surgery (patient F was 10 months postoperative and patient Z was 7 years postoperative) Furthermore the urine draining from the sigmoid pouch was sterile and remained acid throughout the interval of observation Two patients with ileal bladders have also been studied and observed to have no electrolyte disturbances normal BUN and sterile acid urine

Figure 1 compares glomerular filtration rates in the patients with hyperchloremic acidosis and in the patients with no alterations in plasma electrolytes Although glomerular filtration rate was lower than normal in all the patients shown it is noteworthy that the filtration rate was below 20 per cent of normal only in the patients who developed hyperchloremic acidosis This correlation appeared to be consistent regardless of whether the ureters had been transplanted to the intact colon or the skin It was also evident in patients with nephrostomies and in one patient with an intact urinary tract Similar depression of the para amino hippurate clearance was also measured in the patients with hyperchloremic acidosis

The correlation between the intravenous pyelograms and renal function

Various methods for transplantation of the ureters after cystectomy include skin ureterostomies ureterosigmoidostomy with intact anus wet colostomy ileal and cecal bladders and, more recently, the sigmoid urinary pouch[10, 11] as performed according to the technique of Deddish This study was undertaken in an attempt to assess renal function in patients with these various types of urinary diversion and to compare renal function in patients with and without hyperchloremic acidosis The results of this work suggest that renal function was somewhat reduced in all the patients studied who had diversion of the urinary stream In the patients who developed hyper chloremic acidosis however, there was a marked impairment of renal func tion which was preceded by repeated episodes of pyelonephritis or anasto motic stricture

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The correlation between the intravenous pyelograms and renal function

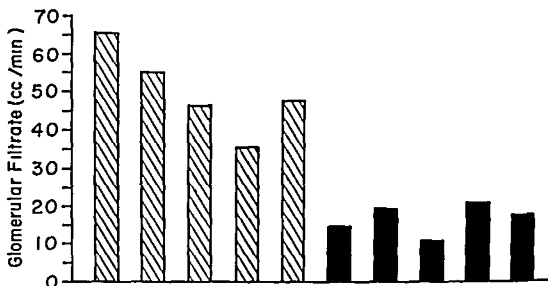


Fig. 1

as measured with inulin and para-amino-hippurate was poor. In many of the patients the reduction in renal clearances was more severe than would have been expected from the interpretation of the intravenous pyelogram. However, all patients with poor concentration of dye on intravenous pyelography always showed marked reduction in glomerular filtration and the clearance of para-amino-hippurate.

DISCUSSION

In the limited number of patients reported, it is evident that hyperchloremic acidosis is seen only in those patients with severe reduction in renal function (10 to 20 per cent of normal). Their renal limitation appears to be secondary to recurrent attacks of pyelonephritis or ureteral obstruction. It is pertinent to note that the situation of hyperchloremic acidosis may develop even with intact urinary tracts or cutaneous ureterostomies if renal function has been sufficiently compromised. However, it is recognized that the incidence is very much higher in patients with urinary diversion to the intact colon. The possibility of ascending pyelonephritis is of course greater in patients with ureterosigmoidostomies above an intact anus where elevated intracolonic pressure secondary to collection and storage of urine in the colon and normal mass contraction of the colon has been shown to cause reflux of fecal bacteria to the renal pelvis.[7] Anastomotic stricture of the ureters would also contribute to impaired renal function. From these observations it would appear that prevention of infection of the urinary tract is an important facet in the prevention of hyperchloremic acidosis following ureteral transplantation. These measures may well require separation of the urinary and fecal streams as well as free drainage to avoid stasis and the bladder substitute should be sterile. Logically the ideal cecal or sigmoid bladder would fulfill these requirements provided that no stricture exists at the ureteral anastomosis. Evidence that this is practical is shown by

the patients presented with sigmoid and ileal bladders and with patent ureters who displayed a sterile urine, and presented no evidence of pyelonephritis or hyperchloremic acidosis The studies of Matheson *et al* on dogs with sigmoid bladders who did not develop hyperchloremic acidosis furnishes additional support for this contention

The evaluation of renal function utilizing intravenous pyelograms would appear to be risky except in severely damaged kidneys where nonvisualization of the urinary tract is usually accompanied by extremely low clearance values The finding of a normal intravenous pyelogram does not however preclude the fact that renal function is severely compromised

SUMMARY

Plasma electrolytes and renal function have been studied in 13 patients with diversion of the urinary tract following cystectomy and in one patient with an intact urinary bladder Frequent episodes of pyelonephritis were found to be followed by marked decreases in renal function and enhanced renal tubular reabsorption of chloride in all the patients who developed hyperchloremic acidosis Patients with sigmoid and ileal bladders are reported and were found to have normal plasma chemistries, moderately reduced renal function and sterile urine It has been postulated that hyperchloremic acidosis and ascending pyelonephritis may be prevented by anastomosing the ureters to an ileal cecal or sigmoid pouch, providing the anastomoses are patent The use of these segments as urinary bladder substitutes should decrease the incidence of ascending pyelonephritis by providing the essential features of separation of the urinary and fecal stream sterile bladder contents and free drainage of urinary contents

REFERENCES

1 Schreiner G E Smith H Kyle L H Renal hyperchloremic acidosis Am J Med *15* 122 1953
2 Boyd J D and Stearns G Concomitance of chronic acidosis with late rickets Am J Dis Child *64* 594 1912
3 Greenspan E M Hyperchloremic acidosis and nephrocalcinosis Arch Int M *83* 271 291 1949
4 Baines G H Barclay J A and Cooke W T Nephrocalcinosis associated with hyperchloremia and low plasma bicarbonate Q J Med Oxf *14* 113 1945
5 Ferris D O and Odel H M Electrolyte pattern of the blood after bilateral uretero sigmoidostomy J Am M Ass *142* 634 641 1950
6 Creevy C D Facts about ureterosigmoidostomy J Am M Ass *151* 120 123 1953
7 Lapides J Mechanism of electrolyte imbalance following ureterosigmoid transplantation Surg Gyn Obst *93* 691 704 1951
8 Matern D I Hypokalemia accompanying hyperchloremic acidosis after ureterosigmoidostomy New England J M *250* 941 944 1954
9 Sherman M S Bone changes following bilateral ureterosigmoidostomy Surg Gyn Obst *97* 150 1953
10 Bisgard J D Substitution of urinary bladder with segment of sigmoid Ann Surg *117* 106 109 1943
11 Bricker E M Bladder substitution after pelvic evisceration Surg Clin N America, *30* 1511 1950

ABSORPTION OF URINARY CONSTITUENTS FROM VARIOUS SEGMENTS OF THE GASTROINTESTINAL TRACT USED AS URINARY CONDUITS OR RESERVOIRS*

JOHN J MURPHY MAUNG KYAW MYINT L B SCOTT AND CLETUS W SCHWEGMAN

The use of various portions of the gastrointestinal tract as reservoirs or conduits for urine has received increasing attention in recent years Many such preparations have proven to be useful clinically and have permitted advances in reconstructive surgery as well as in the field of radical cancer therapy

Complications resulting from the use of bowel for transport or storage of urine have been largely due to renal infection and disturbance of body electrolyte balance Experience has shown that the factor of infection may be controlled in large part by isolation of the segment to be so used from the continuity of the intestinal tract Electrolyte imbalance has been demonstrated to be due to the absorption of urinary constituents in the presence of renal damage[1] The following study was devised in an attempt to elucidate this problem

METHOD

Adult mongrel dogs were used as experimental animals Four types of preparations were studied In the first group of animals both ureters were anastomosed to an isolated segment of distal ileum 12 to 14 cm in length the proximal end of which was closed and the distal end brought out to the abdominal skin to form the stoma of this conduit (Fig 1) An isolated

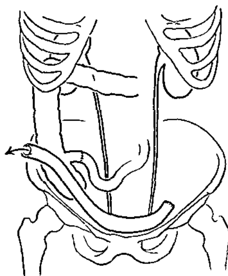


Fig 1 Diagram of ureteroenterostomy to isolated segment of ileum

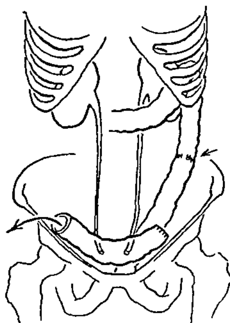


Fig 2 Diagram of ureteroenterostomy to isolated segment of sigmoid colon

*From the Harrison Department of Surgical Research Schools of Medicine University of Pennsylvania Philadelphia Pa

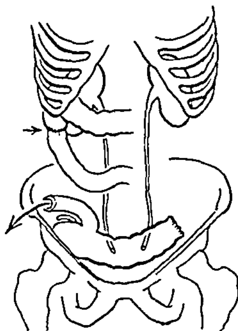


Fig 3 Diagram of ureteroenterostomy to isolated right colon

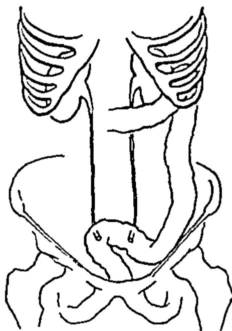


Fig 4 Diagram of ureteroenterostomy to intact sigmoid colon

segment of sigmoid colon was similarly used as a urinary conduit in the second group (Fig 2) In a third group of animals the ureters were anastomosed to a segment of ascending colon 12 to 14 cm in length A short portion of the terminal ileum was used to provide a cutaneous stoma (Fig 3) Ureterosigmoidostomy using the intact sigmoid colon as a reservoir conduit was performed in the fourth group (Fig 4)

Continuous or intermittent catheterization of the reservoir was necessary in the right colon preparations A Penrose drain was placed through the stoma of the ileal and sigmoid conduits for the first 24 hours postoperatively

Two to 21 days after operation the mesentery of the bowel segment to which the ureters were attached was exposed and a sample of blood taken from the largest vein draining the segment At the same time a blood sample was obtained from a peripheral (leg) vein The amount of sodium potassium chloride carbon dioxide combining power and urea nitrogen in each sample was determined by standard laboratory methods

Comparison of the concentration of these substances in the mesenteric and peripheral blood of 3 animals in each group is shown in the following tables

Table 1 reveals that urea was found in significantly higher concentration in the mesenteric blood of the isolated ileal conduit Chloride appeared in slightly higher concentration here and carbon dioxide combining power was very slightly diminished No significant differences were found in sodium and potassium concentrations

Table 2 demonstrates no really significant difference between mesenteric and peripheral blood concentrations of any of the constituents tested in the animals with isolated sigmoid conduits

Table 3 discloses surprisingly high urea concentration in both mesenteric and peripheral blood in the right colon reservoir preparations with a

Table 1 Isolated Ileal Segment Conduit

ANIMAL	BLOOD	Na mEq/l	K mEq/l	Cl mEq/l	CO_2 mEq/l	UN Mg %
#1250	M	138	4.5	92	9	55
	P	137	4.2	89	11	22
#1268	M	142	3.7	113	12	40
	P	144	4.2	101	14	29
#1285	M	138	4.5	114	16	55
	P	138	4.5	109	17	46

Table 2 Isolated Sigmoid Colon Conduit

ANIMAL	BLOOD	Na mEq/l	K mEq/l	Cl mEq/l	CO_2 mEq/l	UN Mg %
#1168	M	152	4.1	105	16	25
	P	152	5.5	109	18	21
#1282	M	132	2.8	106	15	25
	P	132	3.2	103	17	21
#1286	M	144	3.9	117	13	29
	P	142	3.5	114	16	26

Table 3 Right Colon Reservoir

ANIMAL	BLOOD	Na mEq/l	K mEq/l	Cl mEq/l	CO mEq/l	UN Mg %
#1457	M	130	3.7	90	16	68
	P	130	2.8	87	20	60
#1200	M	134	3.2	102	19	58
	P	134	3.7	99	19	52
#1463	M	130	4.8	92	13	84
	P	134	4.4	90	17	73

Table 4 Intact Sigmoid Reservoir

ANIMAL	BLOOD	Na mEq/l	K mEq/l	Cl mEq/l	CO mEq/l	UN Mg %
#1249	M	144	4.7	85	7	52
	P	146	4.7	66	10	38
#1252	M	137	6.6	85	11	47
	P	141	7.7	87	13	24
#1462	M	129	4.3	98	21	59
	P	130	3.6	98	23	40

tendency towards higher levels in the mesenteric blood The significance of this finding will be discussed later Suggestive slight increases in chloride concentration and decrease of carbon dioxide combining power in mesenteric blood are also seen Sodium and potassium levels are respectively similar in each

Table 4 showing the results found in the animals with intact sigmoid reservoirs indicates no striking difference in electrolyte concentration in mesenteric and peripheral blood but a tendency toward higher urea levels is noted with significant increase in this substance in the mesenteric blood Slight diminution of the carbon dioxide combining power in this blood is also apparent

SUMMARY AND DISCUSSION

There appears to be little significant difference in electrolyte absorption in the segments of bowel studied in these experiments Chloride and possibly other acid radicals appear to be absorbed to some extent and approximately equally well by ileum right colon and sigmoid colon Urea is absorbed to a greater extent by all Absorption of all constituents tested was less by the isolated sigmoid conduit than by any other preparation studied

The elevated urea nitrogen levels noted in the reservoir type preparation may indicate the reason for the frequency with which hyperchloremic acidosis is noted in patients with this type of urinary diversion Urinary stasis or back pressure from the reservoir of which this elevated urea nitrogen level is evidence may have an adverse effect upon renal function permitting the development of acidosis The freely draining conduit especially of the sigmoid type would appear to be the method of choice if this complication is to be avoided

REFERENCE

1 Lapides J Mechanism of electrolyte imbalance following ureterosigmoidostomy Surg Gyn Obst *93* 691 1951

METABOLIC ALTERATIONS IN SURGICAL PATIENTS VII THE RELIEF OF LOWER URINARY TRACT OBSTRUCTION*

LESTER PERSKY, JERREL W BENSON, STANLEY LEVEY AND WILLIAM E ABBOTT

The problem of fluid and electrolyte balance in patients with acute lower urinary tract obstruction has been reviewed recently by both Lapides[1] and Moyer and associates The severe salt and water losses which may occur when renal damage exists have been pointed out and the relatively benign course of the average patient has been described Since the metabolic changes in the average patient have not been stressed it seemed worthwhile to do balance studies on a series of unselected patients managed in a routine fashion with common types of obstruction of the urinary bladder resulting from prostatic disease This was undertaken to determine if in the ordinary hospital management of such patients metabolic alterations might occur which would require more than simple attention to an ample fluid intake an adequate outflow and reliance upon clinical impression in regard to the over all physiologic state For this reason a series of patients in acute urinary retention were admitted to the Metabolic Wards of the University Hospitals of Cleveland and balance studies were carried out

METHOD

A series of 6 male patients with urinary retention were studied No criteria other than the existence of vesical obstruction were used in the selection of these patients Routine admission hospital orders were written by the urological service A diet analyzed for nitrogen sodium potassium and chloride analogous to the regular hospital diet in terms of caloric content volume and ionic composition was given to these patients and when possible they were maintained on this regimen during the period of study The diet given these patients contained 1300 to 1800 calories 85 to 95 mEq of sodium 60 to 80 mEq of potassium 90 to 100 mEq of chloride and 8 to 11 gm of nitrogen per day While the amounts of calories and nutrients varied from one patient to another they were kept constant in each individual after the first day Fluid intake was unrestricted In no instance was parenteral therapy employed One liter was added to the determined fluid losses to take into account the insensible fluid loss We believe that this gives a better approximation of the total water balance None of the patients exhibited marked fever or acidosis All excreta were collected upon admission to the ward including the initial catheterized specimen and analyzed for sodium potassium chloride and nitrogen Previously described analytical methods were employed for all excreta and nutrients[3] The length of study varied between 7 and 13 days

Serial blood urea nitrogen and plasma sodium potassium and chloride determinations were made Hematocrit determinations were done on admission and repeated at regular intervals Plasma volumes were also ob

*From the Department of Surgery Western Reserve University School of Medicine and the University Hospitals of Cleveland Cleveland Ohio This work was supported in part by a grant from The National Institutes of Health U S Public Health Service (A 760 (C3) and by the John A Hartford Foundation

tained shortly after admission utilizing the radioactive iodinated albumin technique[4] and repeated at 18 to 72 hour intervals following relief of obstruction. Weights were obtained daily prior to breakfast.

RESULTS

Since the results obtained from these 6 patients all showed similar trends differing only in magnitude and duration of alterations, detailed data will be presented from only 1 representative patient.

C. B. A 69 year old white male (U. H. C. 671 231) entered the University Hospitals of Cleveland for the first time with the chief complaint of difficulty in voiding of 3 years duration. The patient recently had developed dribbling and incontinence. He had no nausea, vomiting, or headache but had noted a diminution in appetite over the past 6 months. There was a 15 lb. weight loss in the 4 months prior to admission. Several weeks prior to being hospitalized he had noted grossly bloody urine. On physical examination he was found to have a distended bladder extending above the umbilicus. The prostate was approximately three times its normal size, rubbery and without nodules. Laboratory data revealed an elevated blood urea nitrogen of 65 mg. per 100 ml., hemoglobin was 7.8 gm. per 100 ml. and an admission urinary specific gravity was 1.008. This patient was studied in the metabolic division for 9 days and ultimately had a suprapubic prostatectomy for benign disease.

The metabolic balances for this patient are shown in Figure 1. In this figure intake and output are plotted on the ordinate and time on the abscissa. Intake is plotted upward from the zero line and output is plotted from the top of the intake column downward. Positive balance is represented by the blank white spaces above the zero line and a negative

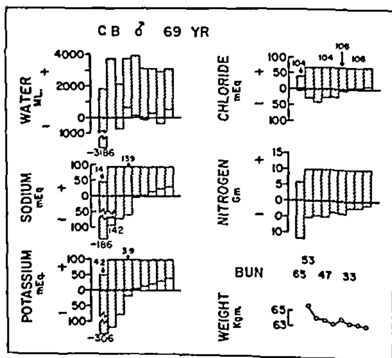


Fig. 1. Metabolic balance studies on patient C. B. following relief of lower urinary tract obstruction due to benign prostatic enlargement. Balances are shown for sodium, potassium, chloride, nitrogen and water.

balance is shown by the extension of the column below the zero line. Plasma electrolyte concentrations are shown by numbers on the appropriate day in the charts.

Although no serious derangement which necessitated special therapy as outlined by others[1, 2] occurred in these patients, certain definite trends can be seen. In the *average* patient fluid requirements are usually met by the patient's own oral intake without need for supplementary parenteral administration. Although initial negative water balances were never completely corrected, they tended to approach balance at the termination of the study.

There was similarly a negative sodium balance initially which in the average case corrected itself readily by the end of 18 to 72 hours. There were no significant changes in plasma sodium concentrations. In the 1 patient with a slightly low plasma sodium concentration salt restriction had been in force due to associated cardiac disease.

Potassium balances were negative regularly at the start of the study and probably represented an unloading phenomenon. This negative balance was eliminated after a short period of constant drainage. Plasma potassium levels showed only slight alterations during the study. Since renal function was not sufficiently disturbed in this group of patients, acidosis, abnormal plasma sodium and potassium concentrations and the wasting of fixed base in the urine were not encountered.

The nitrogen balances behaved as might be anticipated in a group of patients with urinary retention. There were negative balances at the start of each study period similarly reflecting the loss of retained nitrogenous waste products. After drainage was instituted, positive balances were attained again after relatively brief intervals varying from 48 to 72 hours. In 1 patient followed on balance studies during the postoperative period a negative nitrogen balance occurred following operation and persisted for 6 days. In the majority of instances the plasma volumes which were in the expected normal range on admission showed a tendency to decrease slightly after 48 hours.

DISCUSSION

The marked changes in ionic composition, osmolar concentration and extracellular volume which have been so aptly described[1] were not encountered in this series of patients. Certain conclusions appear tenable however in the light of the observations made. Following the institution of drainage in the average patient where obstruction is of relatively brief duration, the kidney function is adequate to maintain homeostasis. The negative balance of water, sodium, potassium and nitrogen observed for the first few days in our patients was readily corrected by drainage. The recognition of patients exhibiting dehydration and sodium and potassium wasting should be apparent if the elevated blood urea does not fall and the acid base balance does not return to normal upon the establishment of an adequate urinary output. Since there was no appreciable abnormality in the plasma volume, dehydration was not thought to exist in these patients. In this group of patients therefore the metabolic studies bear out the clinical observations of urologists that the average patient in retention needs no special therapy and that the kidney's innate reserve function is adequate to restore fluid and electrolyte equilibrium. In such

patients the routine ward diet was adequate to restore imbalances and remedy any deficits It is only in the unusual case that there is need for special measures

It is apparent however that if infection and prolonged retention has occurred, progressive tubular damage with a resultant loss of fixed base could exist The early changes in the kidney produced by lower urinary tract obstruction are readily reversible as evidenced by the data from these patients

SUMMARY

The results of metabolic balance data from 6 patients with urinary tract obstruction due to prostatic enlargement indicate that renal function corrects any imbalances and that no special fluid electrolyte or protein therapy is needed for the *average* patient following the relief of obstruction of short duration

REFERENCES

1 Lapides Jack Physiopathology and therapy of fluid disorders in prostatism Geriatrics *9* 20 27 1954
2 Wilson B Reisman D D., and Moyer C A Fluid balance in the urological patient J Urol Balt *66* 805 815 1951
3 Abbott W E. Krieger H., Babb L. I Levey S and Holden W D Metabolic alterations in surgical patients I The effect of altering the electrolyte carbohydrate and amino acid intake Ann Surg *138* 451-452 1953
4 Storaasli J P Krieger H Friedell H I and Holden W D The use of radioactive iodinated plasma protein in the study of blood volume Surg Gyn Obst *91* 458-464 1950

FUNCTION OF STRIATED MUSCLES IN CONTROL OF URINATION (I) *EFFECT OF PUDENDAL BLOCK**

JACK LAPIDES HOWARD O GRAY AND JOHN C RAWLING

At the present time a marked difference of opinion exists relative to the mechanism of control of micturition One school of thought[1] believes that urination can be initiated as well as inhibited by direct cortical control over contraction of bladder smooth muscle The other group influenced by fluoroscopic[2] and cinefluorographic studies of urination contends that the voluntary mechanism for starting or stopping micturition is mediated entirely through striated (voluntary) muscle

An attempt has been made to conduct experiments which would aid in resolving these diametric theories

In the first part of the study it was elected to determine the role of the muscles of the perineum in urination these include the levator ani external sphincter ani transversus perinei ischiocavernosus bulbocavernosus and the external vesical sphincter muscles

* From the Departments of Surgery University of Michigan Medical School Ann Arbor Michigan and Wayne County General Hospital Eloise Michigan

balance is shown by the extension of the column below the zero line Plasma electrolyte concentrations are shown by numbers on the appropriate day in the charts

Although no serious derangement which necessitated special therapy as outlined by others[1] occurred in these patients certain definite trends can be seen In the *average* patient fluid requirements are usually met by the patient s own oral intake without need for supplementary parenteral administration Although initial negative water balances were never completely corrected they tended to approach balance at the termination of the study

There was similarly a negative sodium balance initially which in the average case corrected itself readily by the end of 48 to 72 hours There were no significant changes in plasma sodium concentrations In the 1 patient with a slightly low plasma sodium concentration salt restriction had been in force due to associated cardiac disease

Potassium balances were negative regularly at the start of the study and probably represented an unloading phenomenon This negative balance was eliminated after a short period of constant drainage Plasma potassium levels showed only slight alterations during the study Since renal function was not sufficiently disturbed in this group of patients acidosis abnormal plasma sodium and potassium concentrations and the wasting of fixed base in the urine were not encountered

The nitrogen balances behaved as might be anticipated in a group of patients with urinary retention There were negative balances at the start of each study period similarly reflecting the loss of retained nitrogenous waste products After drainage was instituted positive balances were attained again after relatively brief intervals varying from 48 to 72 hours In 1 patient followed on balance studies during the postoperative period a negative nitrogen balance occurred following operation and persisted for 6 days In the majority of instances the plasma volumes which were in the expected normal range on admission showed a tendency to decrease slightly after 48 hours

DISCUSSION

The marked changes in ionic composition osmolar concentration and extracellular volume which have been so aptly described[1 2] were not encountered in this series of patients Certain conclusions appear tenable however in the light of the observations made Following the institution of drainage in the average patient where obstruction is of relatively brief duration the kidney function is adequate to maintain homeostasis The negative balance of water sodium potassium and nitrogen observed for the first few days in our patients was readily corrected by drainage The recognition of patients exhibiting dehydration and sodium and potassium wasting should be apparent if the elevated blood urea does not fall and the acid base balance does not return to normal upon the establishment of an adequate urinary output Since there was no appreciable abnormality in the plasma volume dehydration was not thought to exist in these patients In this group of patients therefore the metabolic studies bear out the clinical observations of urologists that the average patient in retention needs no special therapy and that the kidney s innate reserve function is adequate to restore fluid and electrolyte equilibrium In such

FUNCTION OF STRIATED MUSCLE IN CONTROL OF URINATION II *EFFECT OF COMPLETE SKELETAL MUSCLE PARALYSIS*

JACK LAPIDES, ROBERT B SWEET AND LOUIS W LEWIS

Part I of this study demonstrated that micturition can be started and stopped voluntarily despite paralysis of the striated perineal musculature although the voluntary perineal muscles were found to be important in the abrupt inhibition of urination Because some investigators[1] postulate that the diaphragm and abdominal muscles form part of the voluntary mechanism by which voiding is started and stopped all skeletal muscle was paralyzed in order to observe the effect on urination

METHOD

(A) Intravenous D tubocurarine and succinylcholine were used in separate trials to produce the skeletal muscle flaccidity One female and 6 male patients served as subjects they were fully conscious throughout all experiments

Since the effects of the skeletal muscle relaxants on the human autonomic nervous system are not definitely known at the present it was considered necessary to ascertain these effects first Four male patients with *uninhibited neurogenic bladders* were selected as subjects for this part of the experiment These abnormal bladders with uncontrolled contractions resulted from *multiple sclerosis* in 3 patients and from a probable cerebrovascular accident in the fourth Fig 1a demonstrates a normal cystometrograph while Fig 1b illustrates the cystometrograph of a typical *uninhibited neurogenic bladder* Note the uncontrolled contractions of the detrusor produced by filling of the bladder with fluid

Each patient was catheterized and several control cystometric examinations obtained A muscle relaxant was then administered intravenously until flaccidity of all skeletal muscle was evident Artificial respiration

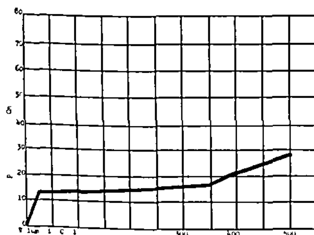


Fig 1a

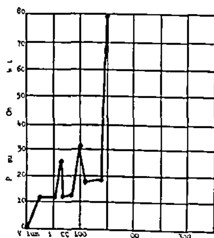


Fig 1b

*From the Departments of Surgery and Anesthesia University of Michigan Medical School and Veterans Administration Hospital Ann Arbor Michigan

METHOD

Since the internal pudendal nerve supplies all of the muscles of the perineum bilateral pudendal nerve blocks were performed to inactivate these muscles Sixteen male and 2 female patients with apparent normal bladder function served as subjects Initially each patient was catheterized and the urinary bladder emptied of its contents 300 ml of physiological saline was then instilled into the bladder and the catheter withdrawn The patient was instructed to stand and cough vigorously Following this the patient was requested to start urination After micturition was well in progress the subject was asked to stop micturition as rapidly as possible The time intervals taken to start and stop urination were noted

Bilateral pudendal nerve block was then accomplished with the patient in the prone position according to the method of Bors[3] Twenty ml of 2 per cent Xylocaine with Alidase (250 units) was injected on each side in the first few patients Because of side reactions the concentration of the Xylocaine was reduced to 1 per cent in the remaining patients The nerve blocks were considered satisfactory if saddle sensation and the bulbocavernosus reflex were absent

After checking the completeness of the pudendal block the patient was subjected to the same testing procedures carried out previously

RESULTS

Prior to nerve block no patient demonstrated stress incontinence on coughing All of the patients were able to stop micturition within 2 seconds after command Fourteen of the subjects (including the 2 females) initiated urination within 2 seconds 3 within 3 seconds and 1 within 4 seconds after request

Following bilateral pudendal nerve block stress incontinence occurred in 1 individual who had been subjected to a previous transurethral prostatectomy The remaining patients were perfectly continent of urine on coughing The ability of the subjects to initiate micturition was not affected by paralysis of the perineal muscles All of the patients could begin urination *within the same time interval recorded prior to nerve block*

However inhibition of urination was affected markedly in most of the patients On command to stop micturition the subject s urinary flow would decrease gradually requiring a period of 8 to 10 seconds for complete cessation whereas prior to block urination could be terminated abruptly within 1 to 2 seconds Several patients were able to inhibit micturition in a normal fashion after apparent complete nerve blockade

CONCLUSIONS

1 Urination can be started and stopped without the aid of the voluntary muscles of the perineum

2 Normal function of the external vesical sphincter and accessory perineal striated muscle is necessary for the voluntary abrupt termination of urination

REFERENCES

1 Langworthy O R Kolb L C and Lewis L G Physiology of Micturition Baltimore The Williams & Wilkins Company 1940

2 Muellner S R., and Fleischner F G Normal and abnormal micturition A study of bladder behavior by means of the fluoroscope J Urol Balt *61*·233 241 1949

3 Bors E Comarr A E and Moulton S H The role of nerve blocks in management of traumatic cord bladders J Urol Balt *63*·653-666 1950

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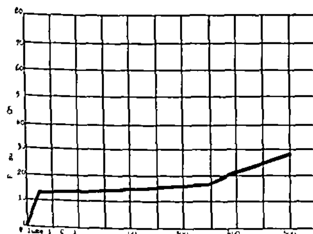


Fig. 1a

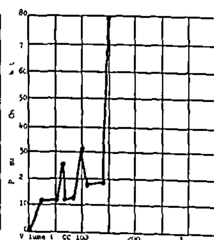


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From the Departments of Surgery and Anesthesia, University of Michigan Medical School and Veterans Administration Hospital, Ann Arbor, Michigan.

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3 Bors E Comarr A E and Moulton S H The role of nerve blocks in management of traumatic cord bladders J Urol Balt 63 [illegible] 1950

Leaking of fluid from the urethral meatus was not observed when the bladder was full during muscle flaccidity

DISCUSSION

The observations made during the present investigation support the concept that micturition is initiated by direct cortical control over bladder smooth muscle

Urination can be stopped by direct cortical control over the detrusor Inhibition of micturition without the aid of skeletal muscle is a relatively slow process The voluntary muscle of the perineum is necessary for the abrupt termination of micturition Urinary continence can be maintained in the absence of tonic activity of skeletal muscle The diaphragm and abdominal muscles are neither necessary for initiation nor inhibition of normal urination

CONCLUSIONS

1 Urination can be initiated and terminated voluntarily without the use of any skeletal muscle in the body

2 Striated muscle is necessary for the rapid abrupt termination of micturition but not for the slow gradual inhibition

REFERENCES

1 Muellner S R and Fleischner F G Normal and abnormal micturition A study of bladder behavior by means of the fluoroscope J Urol Balt 61 233 241 1949
2 Lapides J Observations on normal and abnormal bladder physiology J Urol Balt 70 74 85 1953

THE SURGICAL RECONSTRUCTION OF THE URETER BY A NEW TECHNIQUE*

William C Shoemaker and Robert Bower

Advances in the surgery of replacement in other systems has spurred the search for a satisfactory ureteral substitute So exacting are the normal physiologic functions of the ureter and so prone are artificially constructed ureters to dysfunction that any substitute would of necessity have to be ideal in all respects in order to function for extended periods with acceptable effectiveness The 'perfect' ureteral substitute might be characterized as follows

1 The tissue used must be autologous must have an intact blood supply and must be in plentiful supply

2 The tube must have a lumen adequate for conduction of urine but small enough to discourage reflux

3 It must in its permanent state have a lining of transitional cell type epithelium which is a competent barrier against the absorption of urine solutes and which is not productive of mucous

4 The ureter substitute must be capable of anastomosis to renal pelvis to ureter or to bladder with good healing and low incidence of stricture

*From the Department of Surgery Hahnemann Medical School and Hospital Philadelphia Pa

(intermittent manual compression of rebreathing bag) was necessary because of diaphragm and intercostal muscle paralysis Cystometrographs were repeated during the period of flaccid paralysis Three of the subjects received both *succinylcholine and D tubocurarine on different occasions while* the fourth was given only succinylcholine

(B) When the experiments on the neurogenic bladders had been completed attention was directed toward the 3 patients with normal bladders For several days prior to the experimental run each patient was instructed to practice emptying the bladder in the supine position this was done in order to accustom the patient to voiding in the posture assumed during complete flaccid paralysis On the day of the experimental run the patient was placed in the supine position catheterized and the bladder filled with 300 ml of fluid The catheter was removed and the patient requested to initiate urination and then to stop it After the performance times were noted the patient was paralyzed completely with succinylcholine or D tubocurarine as previously described When complete skeletal flaccidity was present the patient was catheterized again the bladder filled with 300 ml of saline and the catheter withdrawn The patient was asked to initiate and then to inhibit micturition The time intervals taken to carry out the commands were noted

RESULTS

(A) Amounts of succinylcholine and D tubocurarine necessary to produce flaccidity of all striated muscle were insufficient to abolish or even significantly impair the voiding contractions of the *uninhibited neurogenic bladders* of the 4 patients Figure 2 demonstrates the cystometrographs of one of the subjects a is the control cystometrograph and b is the cystometrograph obtained during succinylcholine administration Note that the two graphs are identical

(B) All of the patients with normal bladders were able to initiate micturition within several seconds after command, both before and during skeletal muscle paralysis

Micturition was inhibited by all 3 patients within 2 seconds after request during the control run After administration of succinylcholine or D tubocurarine the subjects required 8 to 10 seconds to stop their urinary stream

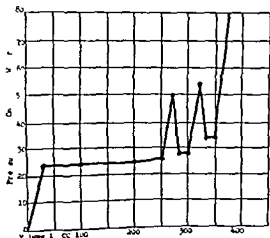


Fig 2a

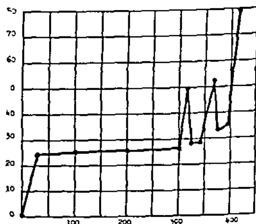


Fig 2b

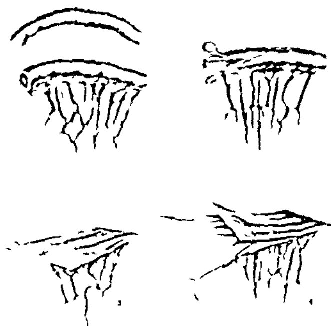


Fig 1a 1 Isolation of intestinal loop and anastomosis of intestinal tract
2 Opening of the isolated loop along the mesenteric border
3 Intestinal loop opened out into a sheet
4 Elevation of mucosa and submucosa from the seromuscular wall

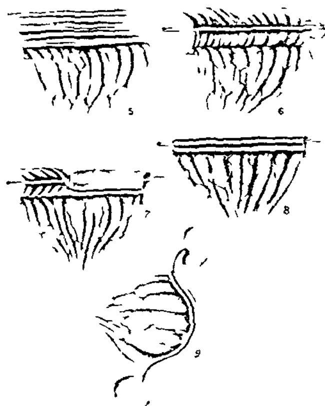


Fig 1b 5 Reversal of the seromuscular graft The serosa now faces the operator
6 Stent placed over the graft and suture begun
7 Graft folded longitudinally over the stent and held with continuous Lembert suture
8 Graft completed
9 Graft in place at the completion of operation

upper anastomosis In 2 instances regenerating ileal mucosa relined the reconstructed ureter Presumably this mucosa arose from an anastomatic site where the submucosa which formed the outer layer of the newly con

Previously reported work by Shoemaker, *et al*[1,2] demonstrated the capacity of transitional cell bladder epithelium to overgrow the serous surface of reversed seromuscular grafts employed for partial bladder replacement and outlined methods for reconstruction of the bladder and ureter

It remained therefore to determine in the first place whether similar overgrowth of transitional epithelium would occur within a long tubular graft and in the second place whether such tubular reversed seromuscular grafts would heal without stricture formation when inserted between renal pelvis and bladder as a ureteral replacement This report is a summary of our findings in this investigation to the present time

METHOD

With the abdomen opened in the midline a segment of mid ileum approximately 20 cm in length was severed from the intestinal tract The intestine was repaired with an end to end anastomosis The bowel was opened longitudinally along the anterior wall close to the mesenteric border The isolated loop was then opened out into a sheet In the first series a cleavage plane between the mucosa and submucosa was defined The mucosa was then dissected free from the submucosa and the seromuscular wall In the second series the cleavage plane was established between the submucosa and the muscularis the mucosa and submucosa were then peeled from the *seromuscular wall At intervals during this procedure* a Pott s clamp or bull dog clamp was placed across the pedicle to decrease blood loss The sero muscular graft was then rolled or folded back over itself to form a tubular structure with the serosa on the inside A continuous Lembert suture of #00 chromic catgut was used to oppose the serosal surfaces over a poly ethylene or polyvinyl tube The plastic stent was made long enough to extend several inches beyond the limits of the graft The ureter was exposed at the vesical junction doubly clamped severed and ligated The ureter was clamped as close to the pelvis of the kidney as possible severed and removed The ureteric vessels were ligated separately

The proximal cut end of the ureter was intubated with the plastic stent The graft was anastomosed to the ureteric remnant or kidney pelvis over this stent with 5 or 6 interrupted sutures of #4 0 chromic catgut

Following the cystotomy the opposite end of the graft was implanted into the bladder using the Nesbit elliptical ureterostomy technique

A #14 dePezzar catheter was placed in the bladder and brought out through the cystotomy incision in males and through the urethra in females The polyethylene (#PE 190) stent was brought out through the dePezzar catheter The cystotomy incision was closed with catgut sutures The abdominal incision was closed in layers with interrupted #00 cotton sutures (Fig 1)

The animals were given parenteral fluids and antibiotics for 1 or 2 days then oral fluids for a similar period and later a full diet as tolerated

RESULTS AND DISCUSSION

In the initial series of 6 dogs the submucosa as well as the seromuscular coat was employed as the graft One animal died less than 12 hours post operatively from the effects of shock and anesthesia The surviving animals were followed from 2 to 7 months In the majority strictures occurred at the

epithelium along the luminal aspect of the reconstructed ureter (Fig 2) Downward growth from the kidney pelvis or proximal ureter while present is somewhat more difficult to demonstrate

SUMMARY

A method for reconstruction of the entire ureter or segments thereof is described together with preliminary observations on the use of this technique in dogs

REFERENCES

1 Shoemaker W C and Marucci H D The experimental use of seromuscular grafts in bladder reconstruction preliminary report J Urol Balt 73 314 19

2 Shoemaker W C Reversed seromuscular grafts in urinary tract reconstruction J Urol Balt (In press)

TOTAL BLADDER SUBSTITUTION USING REVERSED SEROMUSCULAR GRAFTS*

WILLIAM C SHOEMAKER AND PAUL J CROTZINGER

The failure of development of an ideal substitute bladder has been a deterrent to ablative surgery for carcinoma of the bladder The technical bacteriological and physiological difficulties inherent in the problem of bladder substitution though formidable are overshadowed by the importance of its clinical application in the management of carcinoma of the bladder The present communication undertakes to describe the use of the reversed seromuscular graft for bladder substitution following total cystectomy in the experimental animal[1]

METHOD

The peritoneum was opened through a lower abdominal midline incision A 20 cm loop of terminal ileum with its mesentery was isolated and brought down to the region of the bladder The continuity of the gastrointestinal tract was re established by means of an end to end anastomosis using a continuous Connell suture of #2 0 chromic catgut

Following this a longitudinal incision was made approximately 1 cm from the mesenteric border on the anterior surface of the isolated loop The entire loop of bowel then was opened into a sheet By means of sharp and blunt dissection the mucous membrane was separated from the bowel wall Bleeding was controlled with warm moist packs and #3 0 plain catgut ligatures

One end of the isolated loop then was folded onto itself transversely with the serosal side inward The 2 leaves were united to each other along a line 1 cm from the mesenteric border using a continuous suture of #3 0 chromic catgut This formed a hod or trough shaped segment A similar hod was fashioned at the opposite end thus producing a W shaped graft from a long narrow intestinal loop

*From the Department of Surgery Hahnemann Med College and Hospital Philadelphia Pa

structed organ, may have been turned in. Deeply situated islands of mucosal cells which remain on the raw submucosa even after careful dissection and scraping may form a source for growth of ileal mucosa. In our experience growth of ileal mucous membrane will outstrip the transitional epithelium arising from the bladder or ureter.

The overgrowth of ileal mucosa may be prevented in one of several ways. Meticulous attention to an everting Carrel type suture and approximation of the transitional cell mucosa of the bladder or ureter to the inner serosal layer of the substitute will preclude ileal mucosal cells from gaining a foothold. Secondly a method of stripping both the mucosa and submucosa has been devised leaving only the bare muscular coats lined with serosa. This type of graft is much thinner and in the dog more nearly approaches the thickness of the ureter. A second series of 6 animals was attempted using this modification. These animals have been followed for 1½ to 4 months. In no instance was regrowth of ileal mucosa found. One animal died of shock and anesthesia less than 21 hours postoperatively. The operation had been unduly prolonged in this instance.

Exploration of the surviving animals in this latter series from 1 month to 4 months postoperatively revealed a high percentage of stricture at or near the upper anastomosis. It was felt that irritation of the inner serosal surface of the graft by urine producing an inflammatory reaction may eventually result in stricture formation. The use of stents to keep the substitute organ patent for a period of 7 to 11 days apparently does not allow sufficient time for epithelialization with transitional cell lining to take place. Presently work is under way to ascertain if prolonged splinting of the substitute ureter will allow downgrowth of epithelium from the kidney pelvis or ureteric stump as well as upward growth of bladder epithelium and thus prevent stricture formation. Preliminary observations on biopsies of ureters splinted from 1 to 2 months reveal an upward or proximal overgrowth of bladder

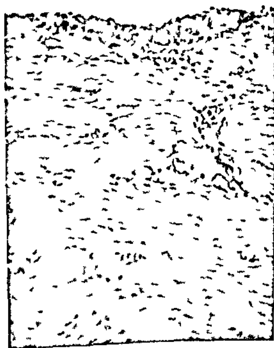


Fig 2 Ureteral graft 31 days postoperatively H and E Medium power

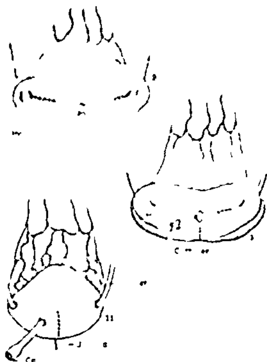


Fig 1(c) 9 Incision in lateral aspect of graft with implantation of ureters
10 The two sides of the graft closed in the midline Catheter placed in graft
11 The completed bladder substitute

day postoperatively 1 on the 13rd day of a stricture of the ureter at the site of implantation with a resultant ureteritis cystica and the third of distemper 3 months postoperatively Three dogs were sacrificed at 2 weeks 9 weeks and 2½ months respectively

Three dogs are living and well at the time of this writing 1 to 10 months after operation The eldest recently delivered a litter of 3 normal puppies

In the initial phases of the project axial implantation of the ureters into the graft was employed This however resulted in a significant number of strictures at the site of implantation Later after the direct elliptical implantation was utilized this annoying complication was less frequent It should be remembered that the tiny size of the ureter in the average sized dog mitigates against reflux but predisposes toward strictures at the implantation site in relative contradistinction to the situation found in man Furthermore there is a definitely limited tolerance of these animals for suprapubic tubes indwelling Foley catheters parenteral and oral medications in additon to repeated blood chemistries and followup examinations

Direct observation of the animals over the entire followup period revealed no urinary frequency incontinence or retention There was no functional disability in any of the dogs in this series Dogs were catheterized at intervals for urinalyses and immediately after voiding on several occasions to test for residual urine Urines were essentially normal and no instance of residual urine was found

Cystoscopy was performed through a #20 panendoscope The mucous membrane was found to be clean smooth and otherwise normal appearing

Intravenous urograms showed essentially normal films except for delayed appearance of dye in one side in each of 2 animals later found to have partial stricture at the site of implantation (v s) retrograde pyelograms were made in a few instances cystograms revealed smooth bladders In 2

Both ureters were clamped, severed and ligated close to the ureterovesical junction and a cystectomy performed In the male the prostate gland and seminal vesicles were excised with bladder The edges of the graft then were trimmed and several mattress sutures of #2 0 chromic catgut were taken to anastomose the graft to the urethra A #14 dePezzar catheter was placed in the urethras of the females and #6 French ureteral catheter in the urethras of the males for a stent for the suture line

The ureters were implanted in the lateral aspect of the substitute bladder by means of the direct elliptical technique of Nesbit The ureters were intubated with #190 polyethylene tubes The 2 hoods, having the general configuration of hemispheres then were united to each other anteriorly in the midline and a #14 dePezzar catheter was placed in the newly constructed bladder anteriorly the polyethylene ureteral catheters were brought out through it The dePezzar and ureteral catheters then were brought out through a stab wound suprapubically and the wound was closed in layers (Fig 1)

Postoperatively, the dogs were kept on parenteral fluids for 24 to 48 hours then oral fluids for another day followed by a regular diet The animals were treated with specific medication only if specific infection ensued

The suprapubic tube and polyethylene ureteral catheters were removed on the 7th or 8th day

RESULTS

Of the 10 dogs subjected to the procedure described previously 1 dog died 10 hours postoperatively of shock and anesthesia Of the 9 remaining dogs 3 died during the followup period 1 of an anastomotic leak on the 12th

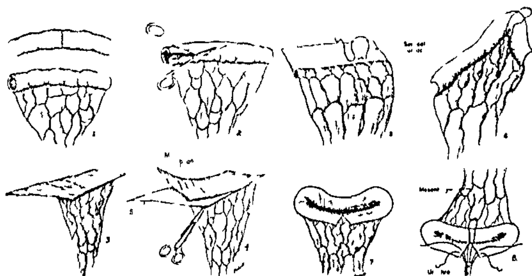


Fig 1 (a) 1 Isolated segment of terminal ileum and reconstituted intestinal tract
2 Opening the ileal segment along the mesenteric border
3 Ileal segment opened out into a sheet
4 Elevation and removal of the mucous membrane
Fig 1 (b) 5 and 6 One end of the graft folded transversely and sutured to form a hood shaped structure with the serosal surface facing inward
7 Similar procedure at opposite end of graft
8 Graft brought down to urethra and anastomosed with mattress sutures

We believe that from these tiny inverted areas along the anastomoses ileal mucous membrane arose and outgrew all competitors until the entire bladder substitute was lined with intestinal mucosa

SUMMARY

1 A method for reconstruction of the bladder following total cystectomy is described

2 A series of dogs subjected to this technique is presented together with preliminary followup studies

REFERENCE

1 Shoemaker W C Reversed seromuscular grafts in urinary tract reconstruction J Urol, Balt (In press)

THE PROTECTIVE EFFECT OF SUBFILTRATION ARTERIAL PRESSURE ON THE KIDNEY*

George C. Morris Jr Charles F Heider and John H Moyer

The production of acute renal failure by hypotension alone is a popular clinical concept However in another investigation we found no renal damage resulted from extended periods of hypotension [1] In these previous experiments on dogs the kidneys were subjected to mean blood pressures below 30 mm Hg during thoracic aortic occlusion Similar resistance to renal damage has been observed in humans who have had surgical procedures requiring occlusion of the thoracic aorta for periods up to one hour In a recent case in which an aneurysm involved both renal arteries it was necessary to occlude these vessels for a period of one hour and forty five minutes The reduction in renal arterial pressure to zero for this time produced severe renal functional impairment Hence it occurred to us that if renal arterial pressures of only 15 to 30 mm Hg might offer extended protection from renal anoxic injury useful clinical application could be found This study compares the effects of renal arterial occlusion with renal arterial hypotension produced by abdominal aortic occlusion The work is an evaluation of subglomerular filtration pressure in the prevention of renal ischemic injury

METHOD

Mongrel female dogs were anesthetized with intravenous pentobarbital (30 mg /kg) after hydrating with water through a gastric tube (40 cc /kg) Control determinations of renal function for both kidneys were then made using averages of three 10 minute collection periods Creatinine was used to measure glomerular filtration rate (GFR) Renal blood flow (RBF) was

* From the Departments of Surgery and Pharmacology Baylor University College of Medicine Houston Texas Supported in part by grants from the Medical Research and Development Board Office of the Surgeon General Department of the Army under contract Number DA-49 007 MD 314 the Houston District Chapter Texas Heart Association and the Cora and Webb Mading Fund for Surgical Research

instances a somewhat heart shaped configuration of the bladder was noted presumably due to the placement of the suture line

Frequent determinations of blood urea nitrogen blood chlorides carbon dioxide combining power serum sodium and and potassium revealed transitory rises in blood urea nitrogen in only 2 animals These 2 animals were later found to have strictures of the ureteral implantation site In no instance was hyperchloremic acidosis or uremia found

Biopsies of sufficient size and depth were taken from the graft area at intervals and necropsy studies were made on all animals which had succumbed and on animals which were sacrificed

While seromuscular reconstructions of bladder which had been subtotally resected uniformly showed replacement with transitional epithelium the histologic picture of the total bladder substitution was by no means constant Several types of changes were noted in the graft area Replacement of inner lining occurred in 4 dogs with a transitional cell epithelium similar to that reported in the subtotally resected bladder series This epithelium Figure 2 however was somewhat suggestive of the epithelium of the urethras or bladder neck Sections taken at the anastomotic line showed areas of apparent overgrowth of the transitional cell epithelium In one animal the serosa of the graft remained intact as such This peritoneal layer had become thickened and in some places infiltrated with inflammatory cells The cells of the innermost layer were small flat and contained a dark staining nucleus

In one instance the seromuscular graft had assumed an inner lining 2 to 3 cell layers thick which suggested metaplasia of the innermost or serosal layer Here it was believed that the cells had taken on epithelial characteristics

In 3 animals a noteworthy occurrence was noted biopsies and necropsy studies revealed ileal mucous membrane completely lining the inner surface of the seromuscular grafts In these animals ileal mucous membrane which had been incompletely peeled off the graft segments had apparently been turned in along the course of the anastomosis between the graft and the urethra



Fig 2 Biopsy of dog No 22 taken 78 days postoperatively H and E High power

We believe that from these tiny inverted areas along the anastomoses ileal mucous membrane arose and outgrew all competitors until the entire bladder substitute was lined with intestinal mucosa

SUMMARY

1 A method for reconstruction of the bladder following total cystectomy is described

2 A series of dogs subjected to this technique is presented together with preliminary followup studies

REFERENCE

1 Shoemaker W C Reversed seromuscular grafts in urinary tract reconstruction J Urol., Balt (In press)

THE PROTECTIVE EFFECT OF SUBFILTRATION ARTERIAL PRESSURE ON THE KIDNEY*

GEORGE C MORRIS JR CHARLES F HEIDER AND JOHN H MOYER

The production of acute renal failure by hypotension alone is a popular clinical concept However in another investigation we found no renal damage resulted from extended periods of hypotension[1] In these previous experiments on dogs the kidneys were subjected to mean blood pressures below 30 mm Hg during thoracic aortic occlusion Similar resistance to renal damage has been observed in humans who have had surgical procedures requiring occlusion of the thoracic aorta for periods up to one hour In a recent case in which an aneurysm involved both renal arteries it was necessary to occlude these vessels for a period of one hour and forty five minutes The reduction in renal arterial pressure to zero for this time produced severe renal functional impairment Hence it occurred to us that if renal arterial pressures of only 15 to 30 mm Hg might offer extended protection from renal anoxic injury useful clinical application could be found This study compares the effects of renal arterial occlusion with renal arterial hypotension produced by abdominal aortic occlusion The work is an evaluation of subglomerular filtration pressure in the prevention of renal ischemic injury

METHOD

Mongrel female dogs were anesthetized with intravenous pentobarbital (30 mg /kg) after hydrating with water through a gastric tube (10 cc /kg) Control determinations of renal function for both kidneys were then made using averages of three 10 minute collection periods Creatinine was used to measure glomerular filtration rate (GFR) Renal blood flow (RBF) was

* From the Departments of Surgery and Pharmacology Baylor University College of Medicine Houston Texas Supported in part by grants from the Medical Research and Development Board Office of the Surgeon General Department of the Army under contract Number DA 49 007 MD 314 the Houston District Chapter Texas Heart Association and the Cora and Webb Mading Fund for Surgical Research

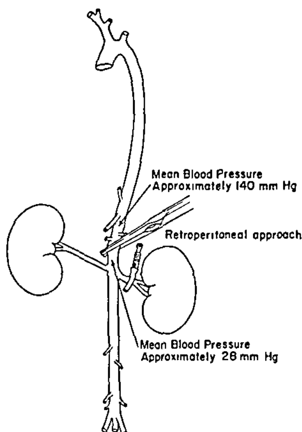


Fig 1 Diagramatic representation of concomitant occlusion of left renal artery with a bulldog clamp and abdominal aorta above origin of renal arteries with a Potts clamp The vessels were exposed through a retroperitoneal left flank incision

derived from renal plasma flow (RPF) measured by para aminohippurate The methods and techniques have been described previously Mean arterial blood pressure was determined by direct femoral arterial manometry Femoral arterial blood was used for all analyses After securing satisfactory control determinations of renal function a left subcostal flank incision was made using aseptic technique The left renal artery and the aorta were exposed through a retroperitoneal approach The origin of both renal arteries was determined and a Potts clamp applied so as to occlude the aorta above both renal arteries The left renal artery was simultaneously occluded with an atraumatic bulldog clamp The aorta and left renal artery occlusion time was 2 hours for dogs 1 through 8 and 2½ hours for dogs 9 through 14 During occlusion the average mean blood pressure in the femoral artery was measured by direct intra arterial manometry On removal of the occluding clamps the left renal artery was checked for return of pulsation The wound was sutured in layers and the dog given intra muscular penicillin *Three to 5 days after operation differential renal* function studies were performed on each kidney to determine any degree of change Each ureter was catheterized with polyethylene tubing and the function of each kidney determined in a manner previously described On completion of the experiment the animals were sacrificed and the kidneys sectioned for gross and microscopic changes

RESULTS

There was a mean reduction in hematocrit from 32 per cent to 25 per cent due to sampling and the operative procedure The mean femoral arterial pressure during aortic occlusion was 25 mm Hg with extremes of 12 to 38

Table 1 The Protective Effects of Subfiltration Arterial Pressure Compared With Complete Ischemia

DOG	WEIGHT kg	HEMATOCRIT		OCCLUSION TIME Hours	FEMORAL MBP DURING AORTIC OCCLUSION mm Hg	GLOMERULAR FILTRATION RATE ml/min			RENAL BLOOD FLOW ml/min		
		C	D			C	D		C	D	
							LK	RK		LK	RK
1	10.0	[illegible]	32	2	20	38	0.2	22	169	2	119
2	16.0	28	2	[illegible]	3	72	3.1	28	403	11	112
3	11.0	20	17	[illegible]	[illegible]	38	0.0	16	181	0	58
4	10.5	37	30	[illegible]	[illegible]	47	0.0	21	295	0	119
5	10.5	31	[illegible]	[illegible]	[illegible]	61	0.0	26	380	0	157
6	13.0	41	31	2	[illegible]	43	12.0	31	256	58	141
7	14.0	40	[illegible]	[illegible]	3	68	0.5	37	368	4	166
8	20.0	[illegible]	[illegible]	[illegible]	38	31	2.0	18	129	8	86
Mean	13.1	34	29		28	51	2.2	25	273	10	120
9	11.0	41	38	2½	12	43	9.0	25	215	47	108
10	14.0	31	33	2½	18	46	0.1	23	235	2	101
11	10.5	41	39	2½	22	38	5.0	20	171	28	121
12	13.5	37	31	2½	21	59	0.0	27	287	0	141
13	18.0	42	34	2½		58	0.0	57	310	0	306
14	14.4	39	39	2½		45	1.0	28	210	0	150
Mean	13.6	39	36		19	48	2.5	30	238	13	15[illegible]
Mean Groups I & II	13.3	36	32		25	50	2.3	27	258	11	135

Foot Notes
C — Control for both kidneys
D — Three to 5 days after left renal artery and proximal abdominal aortic occlusion
LK — Left kidney 3 to 5 days after renal artery occlusion
RK — Right kidney 3 to 5 days after proximal abdominal aortic occlusion
* — Mean Blood Pressure

mm Hg. The mean control glomerular filtration rate for both kidneys was 50 ml/min. The left kidney following renal artery occlusion had a mean glomerular filtration rate of only 2.3 ml/min, a reflection of the severe renal damage which had taken place in all dogs. However the right kidney which had the benefit of subfiltration arterial pressure during occlusion had a post occlusion mean glomerular filtration rate of 27 ml/min. In fact the glomerular filtration rate in 8 dogs exceeded half the bilateral control and none showed significant renal injury. The mean control renal blood flow for both kidneys was 258 ml/min. The left kidney following renal artery occlusion also showed a severe depression in renal blood flow with a mean of only 11 ml/min. The mean blood flow in the right kidney after aortic occlusion was 135 ml/min. The right kidney in 7 of the 14 dogs had blood flows greater than half the bilateral control value.

SUMMARY AND CONCLUSIONS

A technique was developed by which it was possible to evaluate protection from renal anoxic injury which might be offered by subfiltration arterial pressure. The left renal artery and the aorta (above the origin of the renal

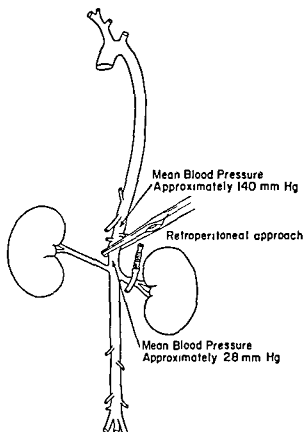


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RESULTS

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Table I The Protective Effects of Subfiltration Arterial Pressure Compared With Complete Ischemia

DOG	WEIGHT kg	HEMATOCRIT C	HEMATOCRIT D	OCCLUSION TIME HOURS	[illegible] PRESSURE WITH OCCLUSION mm Hg	GLOMERULAR FILTRATION RATE ml/min C	GFR D LK	GFR D RK	RENAL BLOOD FLOW ml/min C	RBF D LK	RBF D RK
1	10.0	[illegible]9	[illegible]2	2	20	58	0.2	22	169	2	119
2	16.0	28	2[illegible]	2	*	[illegible]2	3.1	2[illegible]	403	11	112
3	11.0	20	17	2	24	58	0.0	16	184	0	[illegible]8
4	10.5	[illegible]3	[illegible]0	[illegible]	2[illegible]	4[illegible]	0.0	21	27[illegible]	0	119
[illegible]	10.5	31	[illegible]	[illegible]	[illegible]	[illegible]4	0.0	[illegible]	3[illegible]0	0	1[illegible]7
6	15.0	41	34	[illegible]	[illegible]	43	12.0	31	2[illegible]	59	141
[illegible]	14.0	40	[illegible]	[illegible]	3	[illegible]8	0.[illegible]	37	3[illegible]8	4	166
8	20.0	[illegible]3	3	[illegible]	3[illegible]	31	2.0	18	129	8	8[illegible]
Mean	13.1	34	[illegible]		[illegible]8	51	2.2	2[illegible]	273	10	120
9	11.0	41	3[illegible]	2[illegible]	12	43	9.0	2[illegible]	21[illegible]	4[illegible]	10[illegible]
10	14.0	34	33	2[illegible]	18	46	0.1	23	23[illegible]	2	104
11	10.5	41	[illegible]	2[illegible]	22	58	5.0	20	171	28	121
1[illegible]	13.5	3[illegible]	31	2[illegible]	24	59	0.0	27	2[illegible]7	0	141
13	18.0	42	34	2[illegible]		58	0.0	5[illegible]	310	0	30[illegible]
14	14.4	39	39	2[illegible]	[illegible]	4[illegible]	1.0	28	210	0	1[illegible]0
Mean	13.6	39	[illegible]		19	48	2.5	30	238	13	1[illegible]
Mean Groups I & II	13.3	36	32		2[illegible]	50	2.5	27	2[illegible]8	11	13[illegible]

Foot Notes C — Control for both kidneys
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SUMMARY AND CONCLUSIONS

A technique was developed by which it was possible to evaluate protection from renal anoxic injury which might be offered by subfiltration arterial pressure The left renal artery and the aorta (above the origin of the renal

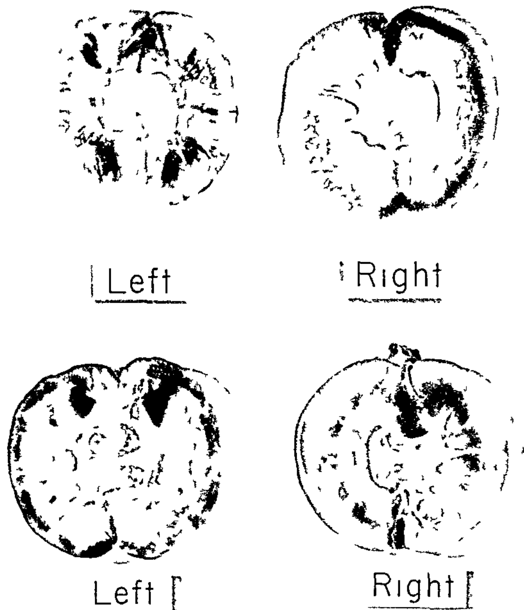


Fig 2 Gross specimens representing characteristic pathologic changes of hemorrhage and necrosis in left kidney subjected to renal artery occlusion The right kidney protected by subfiltration arterial pressure was normal grossly and microscopically

arteries) were clamped for 2 to 2½ hours in 14 dogs Clamping of the proximal abdominal aorta produced a pressure averaging 25 mm Hg in the distal segment Thus the right kidney was exposed to very low arterial pressure while the left was denied all arterial pressure Differential renal function studies were carried out 3 to 5 days later to determine glomerular filtration rate and renal blood flow in each kidney It was found that the kidney protected with a subfiltration pressure developed no functional damage The kidney exposed to renal artery occlusion showed severe functional impairment Further the protected kidney did not lose its compensatory capacity and was able to perform much of the function lost by the contralateral ischemic kidney

REFERENCES

1. Morris, G. C., Moyer, J. H., Cooley, D. A. and Brockman, H. L.: The renal hemodynamic response to hypothermia and to clamping of the thoracic aorta with and without hypothermia. In Surgical Forum 1955. Philadelphia: W. B. Saunders Co., 1955, p. 219.
2. Handley, C. A., Sigafoos, R. and Laforge, M.: Proportional changes in renal tubular reabsorption of dextrose and excretion of p-aminohippurate with changes in glomerular filtration. Am. J. Physiol. 159: 175, 1949.

MECHANISMS CONTROLLING SOLUBILITY OF STONE-FORMING CRYSTALLOIDS*

CORNELIUS W. VERMEULEN AND GEORGE H. MILLER, JR.

It has long been known that the poorly soluble urinary crystalloids are often present in higher concentration than could be obtained in distilled water. Various factors or actions have been brought forward as possible explanations for this apparent supersaturation. Among these are the urinary colloids, total salt effect, and certain specific solubilizing actions such as urea and magnesium upon calcium oxalate. Ordinarily it is assumed that all of the calcium in the urine is freely ionizable and, as a positively charged ion, is available for the formation of one of the poorly soluble stone-forming salts. The possibility exists, however, that a portion is bound to some substance in the urine. If, in normal urine, calcium is indeed bound, the question arises whether there is any difference in the degree of calcium binding in the urine of stone-forming patients.

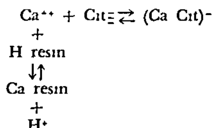
Perhaps the most familiar example of calcium binding is seen when the calcium is complexed with citrate ions. In this circumstance some of the calcium becomes locked up by the formation of a calcium citrate complex, the calcium ion concentration thereby being reduced and the formation of insoluble salts retarded. Since citrate actually is a normal component of urine, one would expect that some degree of calcium citrate complexing might occur.

Whether or not the amount of citrate (or other complexing substance) normally present in urine significantly alters ionic concentration of calcium has been relatively little studied. So far as the authors have been able to ascertain, the only attempt to investigate this matter was made by Flocks[1] and briefly reported in 1950. His method consisted of passing urine through cationic and anionic exchange resin columns and determining the amount of calcium present in the column effluent. He concluded that from 15 to 30 per cent of the calcium in normal urine was bound as a negatively charged complex ion. In the experiments to be described additional observations on this subject have been made through the further use of exchange resins and also rapid dialysis of urine specimens.

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When an ion exchange resin is added to a solution containing a calcium complex the interactions that occur are seen in the following illustrative equation



$$\begin{array}{l} Ca^{++} + Cit^{\equiv} \rightleftarrows (Ca\ Cit)^{-} \\ \quad + \\ H\ resin \\ \quad \downarrow\uparrow \\ Ca\ resin \\ \quad + \\ \quad H^{+} \end{array}$$

In the above formulation both the complexing ion (citrate) and the resin compete for the calcium until equilibrium is established Therefore it is believed preferable to determine the degree of ion complexing by batch rather than column operation when using resins In batch operation the unknown solution is shaken to equilibrium with a given amount of resin rather than allowing the solution to filter through an ion exchange column where conditions are constantly changing as the mixture passes downward In our determinations 15 ml of unknown solution was shaken for 15 minutes on a mechanical shaker with 1 gm of the cation exchange resin (Amberlite IR 120 in the sodium form) Control observations demonstrated that a shaking of 15 minutes was adequate for our purposes though exact equilibrium was doubtless not obtained within that time Calcium complexing in the original solution is indicated by the amount of calcium detectable in the supernatant following shaking with the resin

Unfortunately ion exchange of this type is a reaction of considerable complexity and it is therefore necessary to have rigid control of all conditions From a pure water solution all of the calcium is taken up by the resin Approximately 13 per cent would escape combination with the resin if the test were run on a solution of identical calcium concentration but which contained 1 gm per cent sodium (as sodium chloride) Also it was found that variations in pH markedly altered the amount of detectable complexing Consequently all urines to be tested were prepared in such a way as to contain 15 mg per cent calcium and a total electrolyte content equivalent to 1 gm per cent sodium chloride All tests were also run at a rigidly controlled pH of 6 5 Observations are available on the degree of calcium complexing in 69 normal individuals and 27 patients proved to have urolithiasis A few observations are also available on normal urine that was first passed through a cation exchange column followed by passage through an anion exchange column yielding a final effluent that may be considered as de ionized urine

The results have been expressed as the per cent of calcium remaining in the supernatant after shaking with the resin It must be emphasized that this figure need not actually be the per cent of calcium present as a complex in the original specimen The figures are only of value for comparative purposes since the introduction of a resin inevitably disturbs the equilibrium and the blank reading must be subtracted from it

Data shown in Figure 1 are presented to support the validity of the test employed They show that all but 3 per cent of the calcium is removed from a pure solution when shaken with the resin as described A solution of calcium in salt (1 gm per cent sodium) fails to do so about 13 per cent of

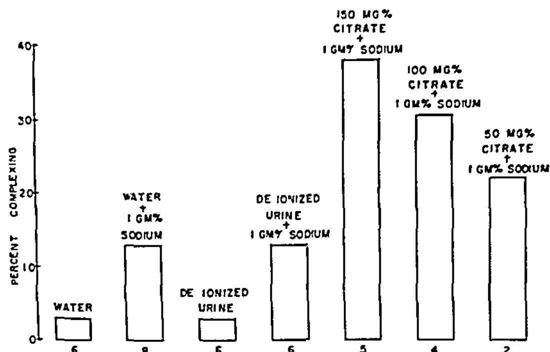


Fig 1 Per cent of complexed calcium detected in known test solutions

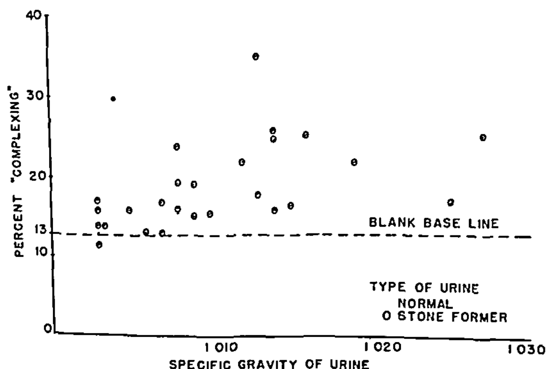


Fig 2 Calcium complexing detected in the urine of 69 normal individuals and 27 patients with urolithiasis

the calcium still remaining in the supernatant When these tests were run using de ionized urine it was found to behave like distilled water The control observations on solutions containing citrate a known calcium complexer, served to show the ability of the test to detect the presence of a complexing agent and that it is capable of distinguishing between different amounts

In Figure 2 is shown the results obtained on the urine specimens on both normal and stone forming individuals It will be remembered that all specimens are actually run with a constant sodium content of 1 gm per cent A blank with water solution containing this amount of sodium gives a figure of 13 per cent which is then the blank base line One can see from the graph that as the specific gravity increases the amount of complexing also increases This is not surprising since more concentrated urine would be likely to contain more complexing agent Both normal and stone forming urine sometimes showed little or no complexing by this test No close correlation was found between the amount of complexing and stone formation and it is not possible from the data to say that calcium complexing is reduced in patients with stone On the contrary a clear cut correlation of calcium complexing with citrate concentration was found in the 17 instances where the urinary citrate concentration was also determined

RESULTS

The results of this study are interpreted as showing that

1 part of the urinary calcium is present in a complexed form thus serving to confirm Flocks in this regard

2 there seems to be no difference between stone formers and normal individuals in the degree of calcium complexing as detected by this test

3 there is a suggestion that the agent to which the calcium is complexed may be citrate but other similarly acting substances are not excluded

It has been suggested that part of the urinary calcium is bound to some other substances as an ionic complex If part of the urinary calcium be bound to some substance that happens to be poorly dialyzable one would expect the freedom of calcium dialysis to be impeded by such a combination To test this possibility the following experiments were all carried out on a rapid dialysis machine over a 2 hours period The water of the bath was changed at half hour intervals and the pH was rigidly controlled with the use of a glass electrode pH meter

A pure urea solution was found to dialyze completely free of urea in the 2 hours period Likewise a calcium chloride solution also was found at the end of 2 hours to be essentially free of calcium If however citrate was present in the calcium chloride solution dialysis of the calcium was incomplete the degree being dependent upon the pH at which dialysis was carried out In another control experiment a solution of calcium chloride to which phosphate was added showed that the addition of phosphate ions did not impair the dialysis of the calcium From these control experiments it became evident that while calcium dialyzed freely if in a pure solution dialysis was impeded if citrate were present the degree of impedance being dependent upon the pH

In Figure 3 the results obtained with urine from normal individuals and those with urolithiasis are given when the dialysis was carried out at a pH

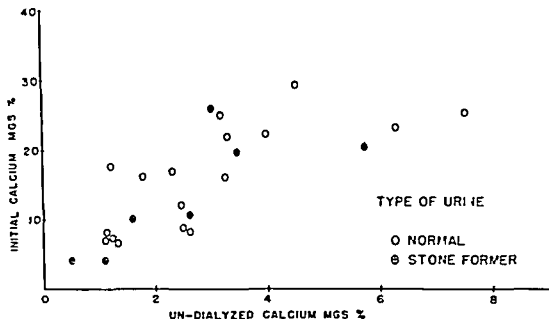


Fig 3 The amount of calcium present in the urine of 17 normal individuals and 7 patients with urolithiasis following a 2 hour dialysis against distilled water

of 6 In every instance part of the calcium remained undialyzed No difference could be detected in the relative freedom of calcium dialysis between normal individuals and stone formers

CONCLUSIONS

From the data presented it is concluded that whereas calcium dialyzes freely from pure solutions its dialysis is impaired from solutions containing citrate a known calcium complexer It is also concluded that calcium is not dialyzed freely from normal urine nor urine of stone formers These findings obtained by quite a different method serve to confirm those presented earlier in this paper that calcium is present in the urine in 2 forms part freely ionizable and part bound

REFERENCE

1 Flocks R H Studies on nature of urinary calcium Its role in calcium urolithiasis. J Urol 64 633 1950

THE RENAL LYMPHATICS AND HYDRONEPHROSIS*

WILLARD E GOODWIN AND JOSEPH J KAUFMAN

In 1949 while working with Sloan and W W Scott on the Trueta shunt one of us noted a marked engorgement of the lymph channels of the kidney associated with stimulation of the renal nerves[1] It was wondered whether or not this apparent increase in lymph formation could help to account for the diminution in urinary output seen under these conditions Carr s recent paper, A New Theory on the Formation of Renal Calculi in which he postulates that renal calculi actually originate in the lymphatics due to formation of microliths which finally erode into the urinary collecting system, has led to renewed interest in renal lymphatic function

The anatomical distribution of renal lymphatics has been extensively studied by Rawson[3] and many others but except for the work of Schmidt and Hayman[4] little has been reported concerning the physiological importance of lymph formation in the kidneys They described experiments indicating that there was an increase of renal lymph production under conditions of diuresis Renal lymph flows of a solitary kidney were calculated to be from 0 6 to 3 2 cc per minute in the dog

We have wondered if ureteral obstruction also may increase lymph flow *and thus fit in with Carr s idea that some renal calculi actually may form* first as lymphatic calculi in renal lymph channels This idea if true would integrate with the common knowledge that urologists share concerning the role of obstruction as one of the factors predisposing to calculus formation

Our present concept is that the renal lymphatics act as a kind of safety valve an additional circulation for the kidney to take care of excess renal fluid during diuresis and under conditions of ureteral obstruction When ureteral obstruction exists there is a considerable increase in formation of renal lymph with a significant return of this fluid into the general circulation This in addition to Hinman s pyelovenous backflow may be viewed as one of the protective mechanisms of the kidney by which it is prevented from destroying itself when urinary obstruction occurs If this function of the renal lymphatics is also important in the absence of obstruction the implications of this mechanism in terms of other renal disease including nephrosis oliguria renal acidosis and nephrocalcinosis nephritis edema states pyelonephritis and chyluria are intriguing

CLINICAL OBSERVATIONS

Some observations which may apply in this respect are as follows

1 A patient with long standing unilateral hydronephrosis showed a contralateral excretory pyelogram after injection of Neo Iopax by needle puncture into the hydronephrotic kidney Was this due to pyelovenous or pyelolymphatic absorption?

2 A patient developed spontaneous rupture of the left kidney while

*From the Department of Surgery Division of Urology University of California Medical Center and the Wadsworth General Hospital Veterans Administration Center Los Angeles California This work was supported by grants from the Schering Corporation and the Fund for the Advancement of Urology

watching a boxing match on television At first this was thought to be a case of Yoo-Yoo's disease[5] Surgical exploration and biopsy of the peri ureteral area revealed ureteral obstruction due to metastatic gastric carcinoma which was previously unsuspected Later an autopsy demonstrated extensive involvement of the abdominal lymphatics with the tumor Did the spontaneous rupture occur because the hydronephrosis in this case could not be relieved by the usual lymphatic safety valve in the presence of lymphatic obstruction by tumor

3 In a patient recently seen with chyluria the retrograde pyelogram showed pyelolymphatic backflow and clearly outlined the renal lymphatics on the left side the site of the chyluria When a small amount of sterile India ink solution was injected into the retroperitoneal space in the pre sacral area it rapidly appeared in urine from the left kidney but not in urine from the right (normal) kidney * Is this not a demonstration of wide open renal lymphatic channels with potential flow in either direction due to obstruction of abdominal lymphatics proximal to the renal pedicle

4 A patient was recently seen with multiple tiny parenchymal renal calculi When retrograde pyelography was performed perfect bilateral delineation of the renal lymphatics was obtained Does this help to confirm Carr's theory

EXPERIMENTS

A number of simple preliminary experiments have been undertaken to test the contribution of the kidneys to lymph flow They are all subject to the criticism that lymph was not collected directly from the isolated kidneys however we believe the results are significant and do represent changes in renal lymph production under the conditions of the experiments

Lymph is collected directly from the thoracic duct in one experiment The volume of lymph flow is measured under basal conditions and under conditions of diuresis in the anesthetized dog When diuresis is established with concentrated glucose solution there is an increase in lymph production When partial or complete ureteral occlusion is produced there is a striking increase in volume of thoracic duct lymph This flow drops to baseline or lower than baseline levels after removal of the ureteral obstruction Figure 1 is a graph of such an experiment

In another type of experiment acute hydronephrosis was produced by ureteral ligation Following this a small amount of radioactive Urokon was introduced into the renal pelvis The radioactivity of thoracic duct lymph and a control vascular area were then monitored with a scintillometer Under these conditions the radioactive material appeared in approximately the same time and concentration in both thoracic duct lymph and in the control vascular area These results were interpreted as showing pyelovenous *and* pyelolymphatic backflow

Hydronephrosis was produced in another experiment by ureteral ligation 24 hours before the isotope experiment † When a measured volume of urine was withdrawn from the hydronephrotic kidney and replaced with

*These studies were performed by Dr Harry DeHaven

†Experiments with radioisotopes were done with the help of Dr Chester A Winter

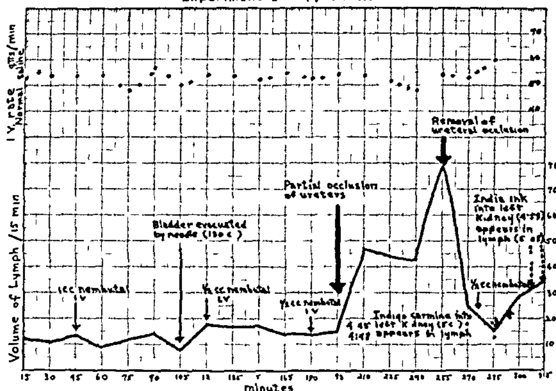


Fig 1 Experiment showing effect of ureteral obstruction on lymph production The broken line at top shows intravenous infusion rate The solid line shows lymph volume collected from the thoracic duct at 15 minute intervals There is a marked increase at 195 minutes when both ureters are partially occluded When the ureteral occlusion is removed at 255 minutes there is a prompt drop of lymph volume output

radioactive Diodrast containing I^{131} it was noted that in 30 to 60 minutes there was a sharp peak of radioactivity in the thoracic duct lymph whereas the blood showed little or none This was interpreted as showing pyelo lymphatic backflow from the kidney

It would seem from these simple experiments that renal lymph may be elaborated from renal interstitial fluid before it becomes urine in the renal pelvis (Experiment #1 above) Also under circumstances of chronic ureteral obstruction it may originate from some connection between the contents of the renal pelvis and the renal lymphatic system (Experiment #3 above)

DISCUSSION

The function of renal lymphatics as a safety valve regulatory mechanism in renal fluid balance may be more important than has been widely recognized in the past This may have application in a variety of renal diseases (e g nephrosis and nephritis) It is undoubtedly of importance in hydronephrosis and some types of pyelonephritis It may be important in the formation of some types of renal calculi Little has been said about this in relation to renal homotransplantation but renal lymph production without appropriate channels for its removal may be one of the causes for failure of renal homotransplantation Further experiments are in progress to try to elucidate some of these points

SUMMARY

The importance of renal lymph formation and safety valve function of renal lymphatics is examined and discussed particularly in relation to ureteral obstruction Preliminary experiments are described in which it is shown that an increase of lymph formation takes place in association with hydronephrosis It is postulated that the renal lymphatics act as a second circulatory system of the kidney and a kind of safety valve under certain circumstances i e, diuresis and hydronephrosis The possible importance of this in disease states is discussed

REFERENCES

1 Goodwin W E Sloan R D and Scott W W The Trueta renal vascular shunt J Urol Balt 61 1010 1027 1949
2 Carr R J A new theory on the formation of renal calculi Brit J Urol 26 105 117 1954
3 Rawson A J Distribution of the lymphatics of the human kidney as shown in the case of carcinomatous permeation Arch Path Clin 47 283 292 1949
4 Schmidt C F and Hayman J M A note upon lymph formation in the dogs kidney and the effect of certain diuretics upon it Am J Physiol 91 157 160 1929 30
5 Bierman H R Yoo Yoos disease N England J M 252 274 275 1955

THE EFFECT OF URETERAL ANASTOMOSIS UPON CONDUCTION OF PERISTALTIC WAVES*

An Electro Ureterographic Study

Harvey R Butcher Jr and William Sleator Jr

Edema and stricture are considered to be the salient if not the sole causes for postanastomotic ureteropelvic dilatation However the relatively short duration of postoperative edema and the progressive quality of cicatricial stenosis make these factors inadequate to explain the frequent disappearance of hydronephrosis months after ureteral anastomosis[1]

This study of ureteral peristalsis by electro ureterography was undertaken in an attempt to find a physiologic explanation for postanastomotic hydroureter and hydronephrosis

METHOD

Adult mongrel dogs were anesthetized by the intravenous injection of sodium pentobarbital (0 03 gm /kg) Under sterile conditions the right midureter of each dog was transected and an immediate mucosa to mucosa single layer anastomosis performed with interrupted sutures of #5 0 chromacized catgut A single arterial silk suture was placed in the suture line to allow its subsequent exact identification No ureteral splint was used

Ureteral peristaltic waves were stimulated by intraluminal balloon distension in the ureteropelvic region after lapsed intervals of 0 to 160 days A multi channeled modified Offner dynograph recorded the action potentials of these waves from intraluminal electrodes of fine silver wire

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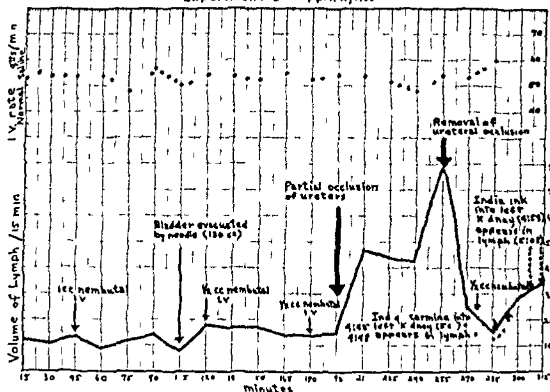


Fig. 1. Experiment showing effect of ureteral obstruction on lymph production. The broken line at top shows intravenous infusion rate. The solid line shows lymph volume collected from the thoracic duct at 15 minute intervals. There is a marked increase at 195 *minutes when both ureters are partially occluded. When the ureteral occlusion is removed* at 255 minutes there is a prompt drop of lymph volume output.

radioactive Diodrast containing I^{131}, it was noted that in 30 to 60 minutes there was a sharp peak of radioactivity in the thoracic duct lymph whereas the blood showed little or none. This was interpreted as showing pyelolymphatic backflow from the kidney.

It would seem from these simple experiments that renal lymph may be elaborated from renal interstitial fluid before it becomes urine in the renal pelvis (Experiment #1 above). Also under circumstances of chronic ureteral obstruction it may originate from some connection between the contents of the renal pelvis and the renal lymphatic system (Experiment #3 above).

DISCUSSION

The function of renal lymphatics as a safety valve regulatory mechanism in renal fluid balance may be more important than has been widely recognized in the past. This may have application in a variety of renal diseases (e.g. nephrosis and nephritis). It is undoubtedly of importance in hydronephrosis and some types of pyelonephritis. It may be important in the formation of some types of renal calculi. Little has been said about this in relation to renal homotransplantation but renal lymph production without appropriate channels for its removal may be one of the causes for failure of renal homotransplantation. Further experiments are in progress to try to elucidate some of these points.

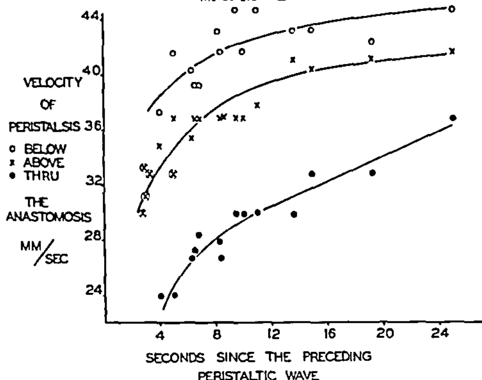


Fig 2 This graph shows the peristaltic wave velocity to be consistently less in a moderately dilated ureter above an anastomosis than in the ureter of normal caliber below it The further velocity decrement for each wave through the region of the anastomosis is caused by a delay of wave conduction at it (Fig 3) Five peristaltic waves in the upper ureteral segment (whose velocities are indicated by ⊗) were preceded by a short inter peristaltic wave interval and did not conduct through the anastomosis

more days after the anastomosis was made Conduction of peristalsis was slower in the ureter above the anastomosis than below it when a definite hydro-ureter was present (Fig 2) However with minimal hydroureter the difference in velocity above and below the anastomosis almost disappeared The difference in velocity of waves above and below an anastomosis increased nearly linearly as the circumference of the ureter above it

The interval required for passage of peristaltic action potentials between the electrodes immediately above and below the anastomosis was prolonged when hydroureter existed This was caused by a delay in conduction of peristalsis at the anastomosis That is there was no significant change in velocity as the wave approached or receded from the anastomosis This was shown by records taken from closely spaced electrodes immediately adjacent to the anastomosis (Fig 3) In 4 dogs an electrode was fortuitously positioned in the anastomosis so that the recording from it showed the arrival of the peristaltic action potential from above a measurable delay and its subsequent departure from the electrode (Fig 3) The duration of the conduction delay at the anastomosis was conversely related to the interval since the arrival of the preceding peristaltic wave In other words within the limits of anastomotic conduction the greater the frequency of peristaltic waves or the shorter the interperistaltic wave

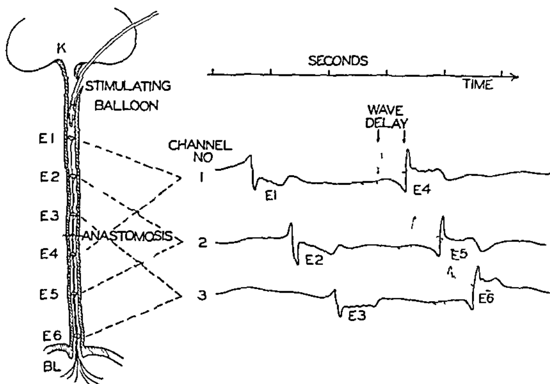


Fig 1 The drawing on the left illustrates the intraluminal position of the electrode assembly and the stimulating balloon during electro ureterography The standard method of recording the action potentials from these electrodes is indicated by the dotted lines leading from each electrode to its respective recording channel Traced action potentials of a single peristaltic wave are shown on the right beneath the time scale A delay in conduction of the peristaltic wave of 0 15 seconds was present in the region of the anastomosis (between electrodes 3 and 4) The dotted waves preceding the actually recorded ones at E4 E5 and E6 signify the position in time these waves would have if no anastomotic conduction delay existed

(0 01 inch diameter) - (Fig 1) The electrode assembly was inserted into the ureter through the ureterovesical orifice after cystostomy The electrodes to be positioned in the ureteral lumen above the anastomosis were passed through it in all instances All electrode leads were insulated by stretching polyethylene tubing (0 04 inches diameter) over them

The extent of proximal ureteral dilatation was determined by intravenous pyelography prior to electro ureterography The width of the longitudinally opened ureter was measured above below and at the anastomosis after completion of the electrical study Multiple tissue sections through each anastomotic site were studied microscopically

RESULTS

Peristaltic action potentials above and below the anastomotic line were usually of normal contour and voltage The voltage of the action potentials was below normal above the anastomosis when poor electrode contact existed in the dilated upper ureter Stimulation of peristalsis by intraluminal distension was performed with equal ease in either segment

Conduction of peristaltic waves across the anastomosis did not occur in the canine ureters studied immediately 7 18 or 21 days postoperatively However with one exception some peristaltic waves traversed it 28 or

interval the longer the delay of wave conduction at the anastomosis Also the longer the average delay in peristaltic wave conduction at the anastomosis the less the number of peristaltic waves conducting through it at a given frequency and the greater the upper ureteral dilatation (Fig 1) In the absence of hydroureter above a healed anastomosis significant delay of peristaltic conduction occurred only during the period of reduced wave velocity[2] The duration of anastomotic conduction delay was independent of the postoperative interval prior to electroureterography

Marked stenosis or physical ureteral obstruction was absent at the ureteral anastomoses which conducted peristalsis The electrode assembly passed through all of these anastomoses with ease

A study of multiple tissue sections made from each anastomosis showed that the extent of the fibrosis and chronic inflammatory infiltration interrupting the continuity of the muscular layers of the ureter correlated with the duration of conduction delay at the anastomoses where conduction existed and was most marked at the anastomoses where conduction did not occur

DISCUSSION

In these experiments an organic ureteral obstruction was not requisite for the genesis or hydronephrosis A functional defect in the peristaltic conduction mechanism at the anastomosis is capable of initiation of hydronephrosis in ureters having no significant narrowing of the lumen at the site of the anastomosis However this does not gainsay the fact that permanent or progressive hydronephrosis may be related to cicatricial stenosis Actually the mechanism of action of the cicatrix may be the prevention of restoration of an adequate smooth muscle bridge for the conduction of peristalsis across the anastomosis Serial tissue sections of one of the anastomoses conducting peristalsis showed smooth muscle bundles continuing through the anastomotic ureteral scar It is reasonable to assume that fibrosis secondary to local inflammation may be of sufficient amount to prevent adequate regeneration in some instances but insufficient to prevent it in others If adequate bridging does not take place then conduction delay will continue to exist and the hydroureter may then be permanent and even progressive though the anastomosis be wide open

REFERENCES

1 Bricker E M Butcher H R Jr and McAfee C A Late results of bladder substitutions with isolated ileal segments Surg Gyn Obst *99* 469-482 1954
2 Sleator W Jr and Butcher H R Jr Action potentials and pressure changes in ureteral peristaltic waves Am J Physiol *180* 261 276 1955

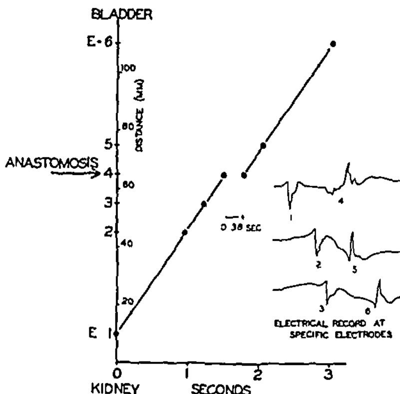


Fig. 3 This graph is a plot of the position of a peristaltic action potential along the ureter in relationship to time. The electrical record of this wave is shown on the right. Electrode 4 was positioned in the anastomosis so that the recording from it showed the arrival, the delay and the departure of the wave. Electrodes 2, 3, 4 and 5 were 10 mm. apart. The slope of the line does not change as it approaches or recedes from electrode 4. In other words, there was no measurable change in the velocity of the wave as it approached or receded from the anastomosis.

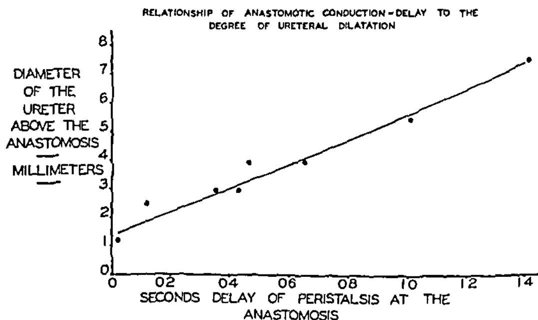


Fig. 4 This graph shows the nearly linear relationship of ureteral diameter above the anastomosis to the duration of delay of peristalsis at the anastomosis.

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